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CHANGES IN IMMUNOLOGIC PARAMETERS AFTER EXPERIMENTAL SPLENECTOMY AND REIMPLANTATION OF SPLENIC FRAGMENTS

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KEY WORDS: spleen; autografting; staphylococcal infection; splenectomy.

Removal of the spleen after trauma and during reconstructive operations on the abdominal organs is an essential stage of the surgical operation. However, frequently splenectomy is followed by postoperative suppurative complications and leads to weakening of the bactericidal properties of the phagocytes and to a fall in the opsonin level, so that susceptibility to infection is increased [4, 5, 7].

These facts have necessitated a search for ways of compensating the disturbed functions after removal of the spleen, and one such way is to reimplant its fragments [1].

The aim of this investigation was an experimental study of the effect of splenectomy and subsequent autografting of the splenic fragments on immunologic parameters in nonlethal generalized staphylococcal infection.

EXPERIMENTAL METHOD

Experiments were carried out on 70 guinea pigs weighing 300-350 g. Depending on the character of the operation the animals were divided into five groups: 1) mock operation (n = 10); 2) splenectomy and autografting of the splenic fragments (n = 20), 3) splenectomy with resection of the pancreas and reimplantation of splenic tissue (n = 20), 4) splenectomy alone (n = 10), 5) splenectomy and resection of the pancreas (n = 10).

The operation was performed with observance of all the rules of asepsis and antisepsis. Animals of all groups were anesthetized with 1% hexobarbital solution (4 mg/kg) and 0.5% relanium* (0.1 ml/100 mg) by intraperitoneal injection. Laparotomy through a midline incision was performed on the animals of group 1 and the hilus of the spleen dissected; the operation ended by suture of the anterior abdominal wall. After removal of the spleen in the animals of group 2 its fragments were reimplanted by the method described previously [1]. In the animals of group 3 splenectomy was combined with distal resection of the pancreas, followed by autografting of the splenic fragments. Splenectomy alone was performed on the animals of group 4; in the animals of group 5 splenectomy was combined with distal resection of the pancreas. The animals were infected intra-

*Diazepam.

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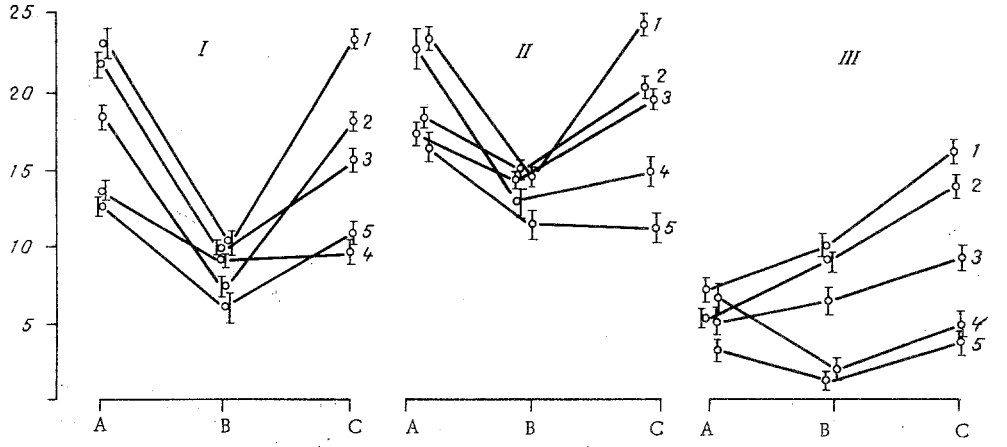

Fig. 1. Time course of immunologic parameters in guinea pigs before and after infection. Abscissa, time of investigation of animals: A) before infection, B) 3 days after infection, C) 9 days after infection; ordinate, percentage of active T lymphocytes (I), of lymphocytes with receptors for staphylococci (II), and of neutrophils with receptors for complement (III). 1-5) Groups of animals.

muscularly with a nonlethal dose (10^{10} bacterial cells in 1 ml) of Staphylococcus aureus 25-35 days after the operations, which caused local suppuration with subsequent generalization (positive cultures from the spleen). The animals were monitored before infection and on the 3rd and 9th days after infection.

The state of the immune system was evaluated by counting the number of active T lymphocytes [6], B lymphocytes [2], and lymphocytes with receptors for nonpathogenic staphylococci [8], and also the number of neutrophils with receptors for the Fc-fragments of IgG (FcγR) and for complement (CR) [3].

A culture of Staph. aureus in a dose of 10^6 bacterial cells in 1 ml was injected into the heart of some of the animals of groups 2 and 3 40 days after the operation. Seedings were taken from the implanted splenic fragments 3 h after injection on to elective protein-salt agar. Positive results of seeding of the test tissue were expressed in colony-forming units (CFU) of the microorganism per gram of spleen. All the results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The percentage of active T lymphocytes 25-35 days after the operation (before infection) was reduced by one-third in the animals of groups 4 and 5, whereas in the animals of groups 2 and 3 the number of active T lymphocytes remained at the characteristic level for animals of group 1 (control). Consequently, splenectomy, alone or combined with resection of the pancreas, itself reduced the percentage of active T cells, and autografting of splenic fragments promoted restoration of these parameters to their initial level (Fig. 1). On the 3rd day after infection a decrease in the relative percentage of active T cells was observed in animals of all the groups. This same picture was characteristic of lymphocytes with receptors for staphylococci. The number of neutrophils with CR was reduced only in the animals of groups 4 and 5.

On the 9th day the number of active T cells in the control (group 1) and in the animals of groups 2 and 3 had returned to its initial level, but in the animals of groups 4 and 5 it remained low. A similar trend was observed for lymphocytes with receptors for staphylococci and neutrophils with CR. However, unlike in animals undergoing the mock operation, these parameters on guinea pigs subjected to autografting and resection of the pancreas were depressed. The number of B lymphocytes in animals of all groups showed no significant changes, and this was also true of the number of neutrophils with FcγR [3].

Consequently, tests determining the percentage of active T lymphocytes, lymphocytes with receptors for staphylococci, and neutrophils with CR proved to be most demonstrative. The level of these parameters is influenced not only by the character, but also by the severity of the operations, and this was particularly noticeable in the presence of infection. For instance, despite autografting of splenic tissue, recovery of the test parameters was less marked if an additional surgical operation (resection of the pancreas) was performed.

A milder course of experimental staphylococcal infection was thus observed in guinea pigs undergoing splenectomy followed by autografting of the decapsulated splenic fragments.

Culturing a homogenate of transplanted splenic fragments after 24 h revealed more than $3 \cdot 10^3$ CFU/g of staphylococci, evidence of the commencing vascularization of the graft and, in conjunction with the results of immunologic investigations, of partial restoration of the function of the grafted splenic fragments.

The results are evidence that the technique of autografting of splenic fragments is a promising method of compensating the functions of the organ lost by virtue of the operation.

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PREPARATION AND CHARACTERISTICS OF MONOCLONAL IKO-GM-1 ANTIBODIES

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With the discovery of the important role of the mononuclear phagocytic system (MPS) in specific immunity interest has risen in the cells of this system [1]. This is particularly the case with the study of the surface structures of cells forming MPS, which has become possible mainly thanks to the use of monoclonal antibodies (MA). A large group of MA, detecting antigens on the surface of mononuclear phagocytes, and also cells of the myeloid series, has now been described. Among them four groups of MA can be distinguished.

Group 1 consists of MA detecting differential antigens on MPS cells, group 2 includes antibodies detecting differential antigens of myeloid cells, group 3 includes antibodies detecting common antigens of myeloid cells and macrophages. Group 4 includes MA with an even wider spectrum of specificity, which mark myeloid cells, MPS cells, and cells of other types.

These antibodies are widely used to study surface structures, differentiation, and functional heterogeneity of macrophages and myeloid cells, and also for the differential diagnosis of nonlymphoid leukemias [2]. However, no information on how to obtain MA detecting differential antigens of MPS and myeloid cells could be found in the Soviet literature.

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