IMMUNOMORPHOLOGICAL CHARACTERISTICS OF MESENTERIC LYMPH NODES AND INTESTINAL LYMPHOID NODULES OF ANIMALS REVIVED FROM CLINICAL DEATH

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KEY WORDS: clinical death; mesenteric lymph nodes; lymphoid nodules; translocation of bacteria

In patients recovering from hemorrhagic shock, multiple trauma, and extensive burns, the basic condition is often complicated by superadded infection, caused by enterobacteria [6, 9]. Prolonged bacteriemia has been discovered in terminal states caused by blood loss, fibrillation of the heart, and asphyxia [1, 2]. Another cause of these complications may be infection of the mesenteric lymph nodes by enterobacteria [5] against the background of a secondary immunodeficiency state in the postresuscitation period [3, 4].

The aim of this investigation was to study the immunological state of the mesenteric lymph nodes and lymphoid (Peyer's) nodules of the small intestine during translocation of enterobacteria in animals surviving clinical death from acute blood loss.

EXPERIMENTAL METHOD

Experiments were carried out on 115 noninbred male and female rats weighing 180-230 g. Under superficial ether anesthesia the animals were catheterized in the femoral vein for bleeding. When the volume of blood lost reached 3.8-4.4 ml/100 g body weight, clinical death began. After 5 min in the terminal state the animals were resuscitated by reinfusion of autologous blood, supported by indirect cardiac massage and artificial ventilation of the lungs. Later, 3-6 h and 1, 3, 6-7, 10-14, and 20-21 days after initiation of clinical death, the rats were killed in an atmosphere of carbon dioxide. Under sterile conditions the peritoneal cavity was opened and pieces of spleen, liver, mesenteric lymph nodes (MLN) and Peyer's nodules (PN), the contents and segments of the wall of the small and large intestines, and blood from the heart were removed. Segments of small intestine 8-10 mm long, MLN, and PN for immunomorphological study were fixed in 10% neutral formalin. Some material was fixed in 1% acetic acid solution in 96° cold ethanol. After dehydration in alcohols of increasing strength the material was embedded in paraffin wax. Paraffin sections 4-5 μ thick were stained with hematoxylin and eosin. Immunoglobulin-containing cells were identified by the direct Coons' method. When this test was set up, fluorescent rabbit polyvalent antibodies to rat immunoglobulins were used. The number of immunoglobulin-containing cells in 1 mm^2 of section of lymphoid follicle was counted by means of the LYUMAM I-1 fluorescent microscope with 40× objective. Weighed samples of tissues and intestinal contents (segments of the wall of the intestine were washed beforehand in 10 changes of physiological saline) were homogenized for quantitative and qualitative bacteriological analysis. Tenfold dilutions of homogenates in 0.7% Difco agar were seeded in a volume of 0.1 ml on Endo's medium. Blood from the heart was introduced into test tubes with Hottinger's broth, containing 1% glucose in the ratio of 1:10. Enterobacteria were identified on the basis of biochemical properties, using the Enterotube II system. The results were subjected to statistical analysis by Student's t test.

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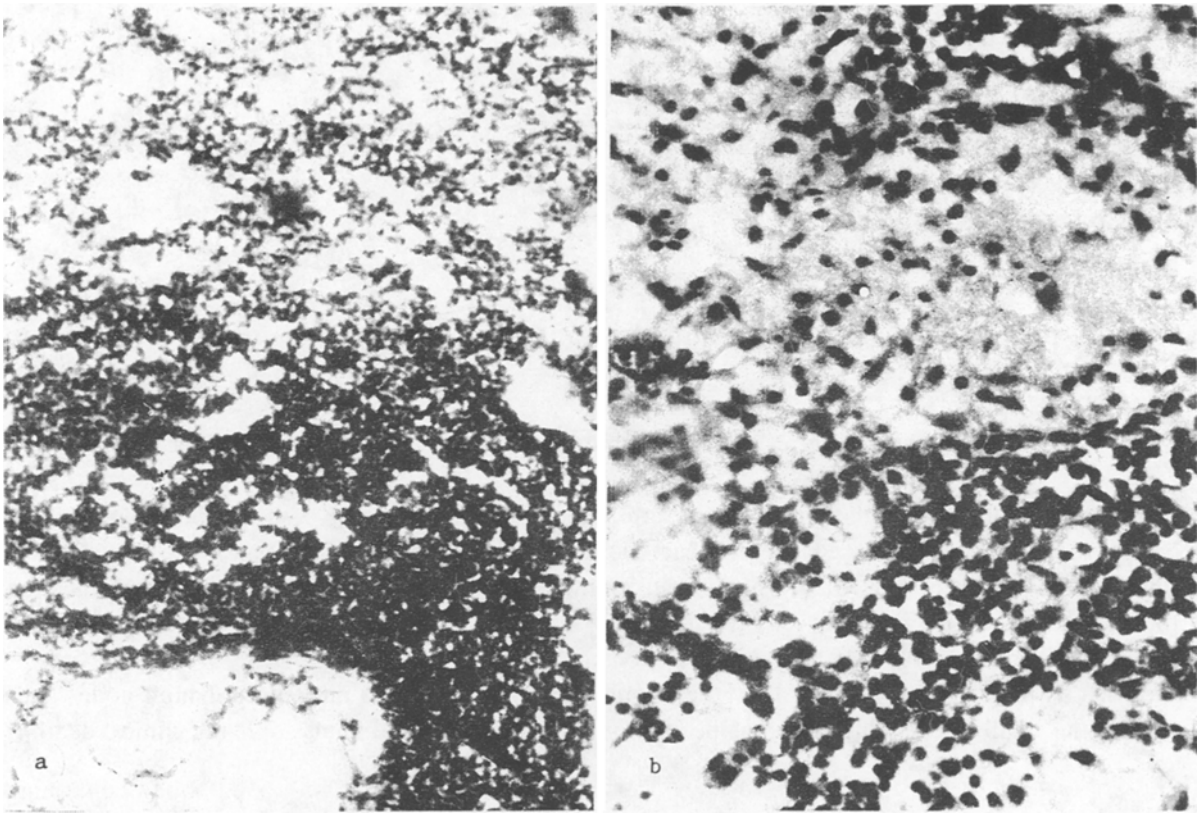

Fig. 1. Morphological changes in mesenteric lymph nodes in early postresuscitation period. a) 3 days after resuscitation (edema of stroma, number of lymphocytes sharply reduced); detail of Fig. la (edema, hemorrhage into medullarly layer). Hematoxylin and eosin. Magnification: a) 50, b) 100.

EXPERIMENTAL RESULTS

The study of histological sections through the small intestine stained with hematoxylin and eosin only 1-3 h after clinical death showed that the cylindrical layer of epithelium, especially on the apices of the villi, was beginning to desquamate, sometimes totally, with "denudation" of the basement membrane. Edema of the villi and diapedesis of erythrocytes were observed. Usually regeneration and restoration of the architectonics of the epithelium were beginning to intensify in the mucous membrane after 1-3 days. In cases when after 3 days the state of the revived animals remained grave, degenerative changes increased in severity, sometimes with the development of focal necrosis of the intestinal wall.

An uneven degree of edema was found in the stroma of the medulla, with a decrease in width of the cortex, 24 h after recovery from clinical death. The boundaries of the structural and functional zones of the lymph nodes were somewhat indistinct. Disturbance of the circulation was manifested as stasis in the microcirculatory bed and hemorrhages in the follicles. Toward the 3rd day of the postresuscitation period perivascular edema of the stroma increased in the cortex and medulla, and foci of hemorrhage appeared (Fig. 1). The follicles were few in number, varied in size, but most frequently seemed enlarged compared with the control. The Peyer's nodules were deficient in lymphocytes, especially in the mantle zone, and the interfollicular spaces, individual areas of which were almost completely devoid of cells. One week after resuscitation only slight edema of the stroma with a little indistinctness of the pattern of the structural and functional zones could be observed. Follicles were solitary, reduced, and without their pale centers. The number of lymphocytes was appreciably increased. Congestion, focal hemorrhages, and moderate edema of follicles were visible in PN during the 1st to 3rd days of the postresuscitation period. At these same times hyperplasia was observed both in the lymphoid follicles and in the interfollicular zone. Toward the end of the 2nd week, in MLN and PN of animals resuscitated from clinical death the histological picture differed only a little from that in the control group.

TABLE 1. Number of Immunoglobulin-Containing Cells of Intestinal Lymphoid Tissue during Postresuscitation Period (M ± m)

Material	Control	Time, days			
		1	3	6—7	13—14
Pia's nodules	100,1±12,2	54,2±4,5*	162,4±26,9	87,1±30,1	99,4±8,7
Mesenteric lymph nodes	254,1±25,9	133,0±10,1*	196,3±22,4	127,4±6,6*	237,3±23,1

Legend. Here and in Table 2: *$p < 0.05$.

TABLE 2. Number of Enterobacteria in Test Material during Postresuscitation Period (CFU/g, log M ± m)

Time of investigation	Contents of large intestine	Wall of small intestine	Pia's nodules	Mesenteric lymph nodes
3—6 h	5,55±0,59	3,93±0,32	3,96±0,26	2,65±0,37
1 day	9,48±0,10*	5,78±0,48*	7,05±0,10*	2,89±0,40
3 day	8,08±0,25*	4,89±0,36	3,15±0,37	2,57±0,12
6—7 days	6,81±0,46	5,16±1,12	5,78±0,39	2,62±0,40
10—14 days	6,73±0,52	4,48±0,00	3,00±0,00	2,99±1,22
20—21 days	4,68±0,27	4,30±0,00	—	—
	5,58±0,26	3,00±0,00	4,24±0,62	—

The results of morphometry of immunoglobulin-containing cells in PN and MLN are given in Table 1. For instance, in MLN during the 1st week of the postresuscitation period the number of immunoglobulin-containing cells fell appreciably compared with the control ($p < 0.05$; Fig. 2). In PN, however, the change in the number of immunoglobulin-containing cells was of a different character. Whereas after 24 h their number was almost reduced by half compared with the control, by the 3rd day, on the contrary, it was 1.5 times greater than initially. Toward the end of the 2nd week of the postresuscitation period the number of immunoglobulin-containing cells both in PN and in MLN was almost the same as in the control group.

During bacteriological investigation enterobacteria were isolated from the liver, spleen, blood, and MLN of the animals starting with 3-6 h and continuing through 3 days of the postresuscitation period. On the 6th-7th and 10th-14th days of the investigation, viable microorganisms could be isolated only from MLN. Translocation of enterobacteria was no longer found 3 weeks after clinical death.

In the course of the postresuscitation period marked changes took place in the frequency of entry of enterobacteria into MLN. After 3-6 h, for instance, translocation was observed in four of 14 experimental animals, toward the end of the 1st day in four of nine, the 3rd day in nine of 19, the 6th-7th days in four of nine, and at the end of the 2nd week, in two of 10 animals.

The population level of enterobacteria 1-3 days after clinical death exceeded the initial values in the contents of the large intestine by 2-3 log units, but in the juxtamural layer of the small intestine by 1.5-2 log units. However, by the end of the 1st week and subsequently, their number fell to the control level (Table 2).

In lymphoid tissue (PN and MLN) compared with the contents or the juxtamural layer of the intestine, no such marked increase in population of enterobacteria was noted. In PN, for instance, not until the end of the 1st day of the postresuscitation period was an increase in their number up to 3 log units observed, and by the 3rd day, the number had returned again to the control level. In MLN, no enterobacteria were present in rats of the control group, whereas in the animals resuscitated, at all times of the investigation they numbered 2.57-2.99 log CFU/g.

Analysis of the results described above indicate persistence of enterobacteria in MLN in the postresuscitation period. Translocation and persistence of perorally introduced bacteria in MLN was demonstrated previously on germ-free animals and animals with antibiotic-decontaminated intestinal microflora [7, 8, 10].

What are the causes of this picture in conventional animals, recovering from clinical death from acute venous blood loss? For instance, if population levels of enterobacteria are compared with contents of the large intestine and the juxtamural layer of the small intestine with the frequency of their translocation into MLN, a connection can be found between these parameters: the higher the population levels of bacteria in the intestine, the more frequently they penetrate

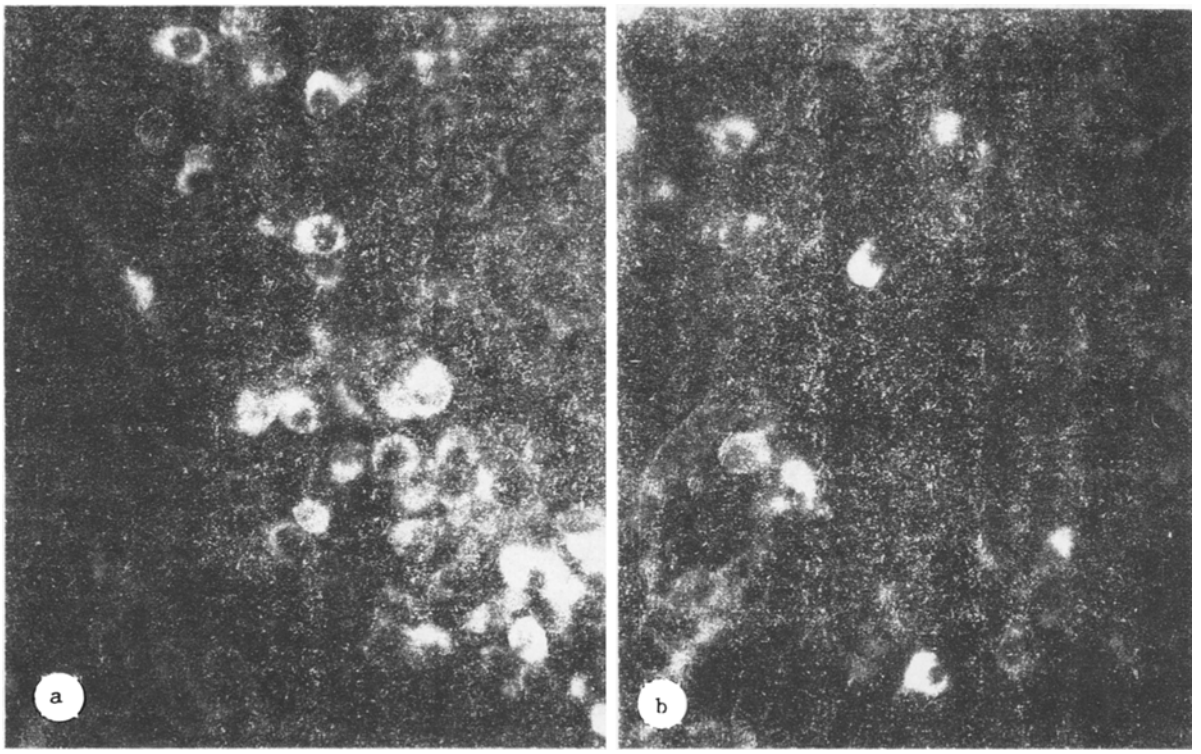

Fig. 2. Changes in number of immunoglobulin-containing cells of mesenteric lymph nodes in early post-resuscitation period: a) control (intrafollicular immunoglobulin-containing cells), b) 3 days later (sharp decrease in number of immunoglobulin-containing cells). Direct immunofluorescence method using fluorescent polyvalent rabbit antibodies to rat immunoglobulins. Magnification: a and b) 250×.

into lymphoid tissue. At the same time, the opposite relationship was found between the frequency of translocation of bacteria and the number of immunoglobulin-containing cells in the regional lymphoid tissue of the intestine of resuscitated animals: the maximal frequency of translocation coincides in time with the minimal number of immunoglobulin-containing cells in MLN.

A threefold increase in the number of immunoglobulin-containing cells in PN was observed only on the 3rd day of the postresuscitation period. Evidently the cause of increased antibody formation was enhanced antigenic stimulation by the increased number of enterobacteria in the intestinal lumen at that time, the region of lymphoid patches. A no less important role in the mechanism of penetration of enterobacteria into the regional lymphoid tissue is the disturbance of structural integrity of the wall of the small intestine discovered in the early postresuscitation period. Pathomorphological changes in MLN (hemorrhages, stasis, edema, reduction of lymphoid tissue, reduction in number of immunoglobulin-containing cells), may also perhaps favor persistence of enterobacteria in them.

To conclude, a high population level of enterobacteria in the intestine promotes their translocation through the damaged mucous membrane into MLN. The pathomorphological changes in the regional lymphoid tissue and inhibition of antibody formation promote maintenance of viability of the enterobacteria in MLN for a period of 2 weeks after resuscitation.

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LONG TERM CHANGES IN THE VASCULAR WALL AFTER LASER RECANALIZATION OF AN ARTERY

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Research into an alternative method of treatment of atherosclerosis of the vessels, namely laser recanalization, has been conducted vigorously in recent years [1, 2, 8, 9, 12, 13]. Experiments in vitro and in vivo have led many research groups to use this new method in clinical practice [1, 2, 5, 9, 10, 11]. Nevertheless, laser recanalization of atherosclerotic arteries still requires further intensive experimental study and analysis of the late results of its clinical application [10, 12, 13], with a view to establishing the place and role of the method in the treatment of arterial atherosclerosis.

The method consists essentially of removing the atherosclerotic plaque from the lumen of an artery by the use of laser energy. Laser radiation is transmitted by thin flexible light guides, advanced to the site of the atherosclerotic lesion in the vessel by the use of special laser catheters, and by means of transcutaneous catheterization of the artery. We have undertaken an important study of repair processes in the arterial wall after laser irradiation, for they determine the patency of the recanalized vessel in both the short and the long term.

The aim of this investigation was to determine experimentally possible alternative forms of the course of repair of the arterial wall after laser application and, on that basis, to choose the optimal regime and tactics for laser recanalization of blood vessels.

EXPERIMENTAL METHOD

In chronic experiments on nine mongrel dogs, laser irradiation was applied to the intact vessel wall by means of different systems of laser catheters. Laser irradiation was applied to the wall of the aorta or iliac arteries through a transcutaneous endovascular access (through the femoral or axillary artery). After induction of anesthesia and identification and catheterization of the artery, aortography or angiography of the iliac arteries was carried out. When the angiographic picture had been obtained the site for laser application was identified on the basis of roentgeno-anatomical data. Next, a guided laser catheter or specially modeled angiographic catheter with light guide was introduced into the lumen of the aorta or iliac arteries. Such catheters enabled laser radiation to be applied to the vessel wall. Manipulations of the laser catheter in the lumen of the vessel were carried out under roentgenotelevision control on an angiographic monitor, making

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