ROLE OF THE SPLEEN IN THE REGULATION OF THROMBOCYTOPOIESIS

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Experiments on dogs showed that splenectomy is followed by thrombocytosis, a decrease in the adhesive and aggregative properties of the platelets, and a decrease in their mean life span. The daily platelet production is increased and a study of the megakaryocytes showed a sharp shift toward mature forms. The number of megakaryocytes in the bone marrow was reduced. It is suggested that a factor controlling differentiation of the precursor cells of megakaryocytes is produced in the spleen.

KEY WORDS: **thrombocytopoiesis**; megakaryocyte; adhesiveness of platelets; splenectomy.

The participation of the normal spleen in hematopoiesis in general and in thrombocytopoiesis in particular has long been a subject for investigation. This is because of the importance of the spleen in hematopoiesis during intrauterine life. The hematopoietic function of the spleen is preserved to some extent in the postnatal period also. However, paradoxical as it may seem, very little is known about the function of the normal spleen [1]. It is now accepted that after removal of the spleen thrombocytosis is observed, but there is no agreement about its mechanism. According to some workers [3, 8-10], a leading factor in this mechanism is removal of the functions of the spleen concerned with the storage and lysis of platelets. Other workers [4] regard the spleen as an organ which inhibits thrombocytopoiesis by a humoral mechanism. Great importance is attached to the production of antiplatelet antibodies in the spleen [7] or the sequestration of platelets in that organ [6]. According to a recent report, releasing factors which may activate megakaryocytopoiesis and may also stimulate the release of platelets into the circulation, are produced in the spleen [13].

The object of this investigation was to study the possible mechanisms of participation of the spleen in thrombocytopoiesis by investigating the state of the megakaryocyte-platelet system and the kinetics of platelets after splenectomy.

EXPERIMENTAL METHOD

Observations were made on eight dogs of both sexes. After a stable background had been established, splenectomy was performed under general morphine–ether anesthesia. Observations were made periodically in the course of a month.

Thrombocytopoiesis was assessed with respect to the following indices: the number of circulating platelets (by the method of Brecher and Cronkite), their adhesiveness (by Wright's method in Baluda's modification) and aggregative properties (by Born's method), the mean life span and daily output of platelets (by the method of Mosyagina and Torubarova), the number of megakaryocytes and myelokaryocytes in 1 μl bone marrow, the differential megakaryocyte count, the glycogen content of the platelets (by Shabadash's method), and their content of acid phosphatase and lipids (by the method of Burstone and McManus, respectively). During the statistical analysis of the results all data were compared with the original indices.

EXPERIMENTAL RESULTS

The experiments showed that from the 2nd day after splenectomy there was a sharp rise in the number of circulating platelets. Their increase reached a maximum during the 2nd week after the operation, and this was followed by a small decline, although by the end of the 1st month the number was still considerably increased.

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TABLE 1. Changes in Number and Functional State of Platelets after Splenectomy (n = 8; M ± m)

Index	Background level	Days after splenectomy			
		2	15	21	30
Number of platelets (thousands/μl)	170±10,8	273±7,8*	325±17,8*	282±18,5*	301±37,5*
Adhesiveness of platelets, %	29±1,1	26±1,0*	23±1,4*	27±2,0	28±3,2
Aggregation index	0,66±0,009	0,42±0,006*	0,43±0,005*	0,44±0,005*	0,47±0,008*
Mean life span of platelets, days	2,8±0,2	1,7±0,2*	1,7±0,14*	2,1±0,05*	2,16±0,08*
Lipid-positive platelets, %	81±2,3	67±3,6*	67±4,9*	60±6,3*	56,6±5,7*
Glycogen-positive platelets, %	73,4±2,5	78±3,6*	81±3,4*	67±2,4*	66,7±3,3
Phosphatase-positive platelets, %	41±3,2	54±4,8*	42±3,3	34±3,8	42,3±4,0

Legend. Here and in Table 2, statistically significant differences from the background level are marked by an asterisk.

During the first 2 weeks after the operation this increase in the number of platelets was accompanied by a decrease in their adhesiveness, but this returned to normal after the 3rd week. After splenectomy there was a tendency for the aggregative power of the platelets to be enhanced. Since these changes occurred in the presence of marked thrombocytosis, which must inevitably have been reflected in the aggregative power of the platelets, it was deemed necessary to calculate the index of the degree of aggregation by dividing the degree of aggregation expressed in millivolts by the number of platelets in thousands. The index calculated in this way represents the partial aggregative power of a precise number of platelets. It was found that the aggregation index fell significantly after splenectomy and remained low for 30 days. From the 2nd day after the operation the mean life span of the platelets also was sharply reduced, and this also remained low for a month (Table 1). When the spleen is removed, an important factor related to the functional integrity of the platelets evidently disappears. In this connection it was interesting to study some cytochemical indices of the platelets reflecting their functional state.

These investigations showed that after a short increase the number of phosphatase-positive platelets gradually fell, and this was particularly noteworthy 3 weeks after splenectomy. At the same time the number of glycogen-positive platelets also was reduced after an initial increase. The lipid content fell immediately after splenectomy and the number of lipid-positive platelets continued to fall in the course of a month (Table 1).

These results, reflecting disturbances of intracellular metabolism in the platelets, emphasize yet again the important role of the spleen in thrombocytopoiesis, namely regulation of metabolism in the platelets before their release into the circulation.

The state of megakaryocytopoiesis also was investigated after splenectomy. It was found that immediately after splenectomy the daily output of platelets was sharply increased and remained high for a month; the differential megakaryocyte count showed a decrease in the number of megakaryoblasts and promegakaryocytes, indicating the more rapid maturation of these cells. From the 2nd day after the operation, in the differential megakaryocyte count the number of free nuclei was increased, evidence of stimulation of megakaryocytopoiesis (Table 2). Counting the megakaryocytes in the bone marrow gave no consistent results. Changes in the number of myelokaryocytes likewise were not significant.

TABLE 2. Changes in Indices of Megakaryocytopoiesis after Splenectomy (n = 8; M ± m)

Index	Background level	Days after splenectomy			
		2	15	21	30
Megakaryoblasts, %	3,8±0,5	1,7±0,04*	0,3±0,2*	0,5±0,2*	—
Promegakaryocytes, %	7,7±0,9	3,8±0,8*	2,0±0,3*	2,0±0,4*	—
Megakaryocytes, %	78,7±1,9	75,3±1,8	79,8±1,4	84,3±0,7*	—
Free nuclei of megakaryocytes, %	9,7±0,9	19,0±2,5*	17,8±1,4*	13,0±0,7*	—
Daily platelet production, thousands	57,6±5,7	101±22,6*	166±11,8*	132,4±17,2*	98,6±6,9*
Number of megakaryocytes, thousands/μl	38,0±7,2	21,0±3,3*	14,0±4,0*	15,0±3,5*	23,5±5,0*
Number of myelokaryocytes, thousands/μl	92,0±20,0	64,0±11,0	70,0±10,3	46,0±11,3*	82±13,0

After splenectomy, besides the marked and prolonged thrombocytosis and the increase in the daily output of platelets, the adhesiveness and aggregative power of the platelets were reduced and their mean lifespan shortened. Intracellular metabolism in the platelets also was disturbed: The decrease in the number of lipid-positive platelets was the most conspicuous feature. This explains the worsening of the functional state of the platelets, for lipids account for about 17% of the dry weight of the platelets and they participate in thromboplastin formation and in the activation of blood coagulation [11, 12], whereas glycogen is the main source of the energy reserves of these cells.

The myelogram showed a sharp shift of the differential megakaryocyte count toward mature forms and a decrease in the number of inactive megakaryocytes. The number of megakaryocytes in 1 μl bone marrow, however, fell significantly from the first few days after splenectomy and remained low for 25-30 days, when the number of circulating platelets was significantly higher than normal. The decrease in the number of myelokaryocytes in the bone marrow was less marked (Table 2).

Only suggestions can be made regarding the mechanisms of interaction between the spleen and bone marrow. Radioisotope studies on mice after splenectomy have not shown any increase in thrombocytopoiesis, although the number of circulating platelets rose [7]. Measurement of the diameter of the platelets after splenectomy showed a decrease, although there was no corresponding change in their total mass; this explains why after splenectomy the activity of the platelets is unchanged [8].

On the basis of comparison of the results of investigation of the number and functional state of the circulating platelets with the results of investigation of the number and functional state of the megakaryocytes after splenectomy it can be postulated that the normal spleen facilitates proliferation and restrains differentiation of the megakaryocytes and their precursors. This is all the more probable because, according to data in the literature [2], the conditions of the microenvironment in the stroma of the spleen are more favorable for proliferation of hematopoietic stem cells in general and the megakaryocyte series in particular. This is further confirmed by the fact that colony-forming units from the spleen lead to growth of a much larger number of megakaryocytes on transfer than those from bone marrow [5]. After splenectomy the conditions for normal differentiation of precursor cells of megakaryocytes are evidently disturbed and conditions are created for the more rapid differentiation and formation of functionally imperfect platelets. Consequently, the normal spleen produces a factor controlling differentiation of precursor cells of megakaryocytes.

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