Paradoxical Effect of Lymphocytes during Filtration of Platelet-Rich Heparin-Treated Plasma

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According to current notions, all blood cells present under any conditions in the microcirculatory system have a more or less expressed inhibitory effect on the blood flow, particularly so in the capillary bed. This is due to the relatively large size of red and white blood cells [7], their low deformability, and the pronounced adhesive and aggregation properties of granulocytes and platelets [2]. We have not yet come across any reports in the relevant literature which doubt this assumption vis-a-vis any conditions of capillary blood flow.

The aim of our research was to elucidate the effect of lymphocytes on the filtration of platelet-rich heparin-treated plasma.

MATERIALS AND METHODS

Blood samples from 21 donors were examined. Blood was collected from the cubital vein on an empty stomach. Richter heparin in a concentration of 10 U/ml blood was used as preserving agent. This agent was chosen because heparin does not block the adhesive or aggregation activities of platelets and other blood cells [3], and this was a necessary condition for our study. Plasma rich in platelets but devoid of all leukocytes was prepared by whole blood centrifugation at 150 g for 25 min (plasma 1). Plasma rich in platelets and lymphocytes but devoid of granulocytes was prepared by whole blood centrifugation at 150 g for 10-15 min (plasma 2). To detect the effect of lymphocytes present in the plasma on platelet block filter capillaries during plasma filtration, plasma 1 and plasma 2 filterabilities were compared. Plasma was filtered through Nuclepore nuclear filters with pore diameter 5 μ at a constant pressure 10^4 Pa for 2 min. Filterability was assessed by the filtered plasma volume. Cell adhesion was examined using nylon threads by a previously described [9] modified [4] method and assessed by the count of adhesive cells (the difference between these cell counts in the initial plasma and in the plasma after contact with nylon) and by the adhesion index (percent ratio of adhesive cell count to total cell count).

RESULTS

The cellular composition and filterability of plasma 1 and plasma 2 are presented in Table 1, platelet and lymphocyte adhesive properties in Table 2. Plasma 2 contained 8 times more lymphocytes than plasma 1. There were virtually no granulocytes in either plasma sample. The platelet count of plasma 2 was 24% higher than that of plasma 1, but the difference was unreliable. The platelet adhesion index of plasma 2 was negligibly and unreliably higher than that of plasma 1. At the

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TABLE 1. Cellular Composition and Filterability of Plasma 1 and Plasma 2

Composition	Plasma	
	1	2
Lymphocytes, $\times 10^9$/liter	0.16±0.02	1.30±0.07*
Granulocytes, $\times 10^9$/liter	0.04±0.01	0.06±0.01
Platelets, $\times 10^9$/liter	358±33	443±36
Filterability, ml	4.73±0.40	8.46±0.34*

Note. Asterisk: reliability of differences ($p<0.001$) from respective plasma 1 parameter.

same time, the count of plasma 2 platelets adhering to nylon threads reliably (by 37%) surpassed this value in plasma 1. Evidently this resulted from both an unreliably higher platelet count and unreliably higher platelet adhesion index of plasma 2. The lymphocyte adhesion index was extremely low: only every eighth lymphocyte adhered to nylon in both plasmas. Still, the difference in the counts of adhesive lymphocytes in the two plasmas was highly reliable, as the total count of lymphocytes was much higher in plasma 2.

All these differences in cellular composition and adhesion resulted in a lower filterability of plasma 2 in comparison with plasma 1. First, the total platelet count was, albeit unreliably, still noticeably higher in plasma 2 than in plasma 1. Second, and this is very important, the count of adhesively active platelets was more than one-third higher in plasma 2, that is, the platelet component of plasma 2 is better predisposed to the filter capillaries platelet block. Third, the presence of many lymphocytes in plasma 2 implies a lower filterability of the plasma because lymphocytes are capable of resisting the capillary flow of the fluid in which they are suspended [5].

But despite all the negative aspects ofg the filterability of plasma 2, it was filtered 80% more intensively than plasma 1, which would have seemed to be preferable in this aspect.

TABLE 2. Adhesion of Platelets and Lymphocytes in Plasma 1 and Plasma 2

Cell adhesion	Plasma	
	1	2
Adhesive platelet count, $\times 10^9$/liter	179±19	245±29*
Platelet adhesion index, %	50.0±5.3	55.3±6.5
Adhesive lymphocyte count, $\times 10^9$/liter	0.02±0.01	0.18±0.02**
Lymphocyte adhesion index, %	12.5±4.0	13.8±1.5

Note. Asterisk: reliability of difference from respective plasma 1 parameter: one asterisk $p<0.05$, two asterisks $p<0.01$.

A crucial difference between plasma 1 and plasma 2 consisted in the virtual absence of lymphocytes in the former and their high content in the latter, and we proceeded from this difference when discussing our paradoxical result. The only route by which lymphocytes might facilitate filtration of platelet-rich plasma that has been described in the literature is connected with the capacity of lymphocytes to inhibit platelet activity [6]. But in our experiments platelet adhesion was not reduced in the presence of lymphocytes, and the number of adhesive platelets was even higher in plasma 2. Evidently plasma 2 platelets adhered to filter capillary walls somewhat more, not less, intensively than plasma 1 platelets. Unfortunately, we did not investigate platelet aggregation activity, which is responsible for the subsequent formation of platelet blocks in filter capillaries. Maybe it is this component that mediates the effect of lymphocytes on plasma filtration. But platelet adhesion and aggregation are closely related and it is hardly likely that they change in different directions. Still, this problem needs to be researched.

On the other hand, the mechanism of better filtration of plasma 2 through 5 μ filter capillaries may be explained not by differences in platelet block formation but by differences in the destruction of these blocks in filter capillaries in comparison with plasma 1. Lymphocytes appear to be the only factor influencing this process. Theoretically, even simple mechanical destruction of intracapillary platelet blocks by lymphocytes is possible. Lymphocytes are not characterized by marked adhesive activity (Table 2), at least in comparison with platelets of granulocytes [1,8]. Their round, poorly deformable nuclei are just slightly smaller in diameter (4.5 μ [7]) than the lumen of the filter capillary and the extremely folded cell membrane [2] is not rigid and permits the cell transverse diameter to be altered to acquire almost the nuclear diameter. Thus, for a sufficiently high perfusion pressure it may be possible for the lymphocytes to move along the capillary lumen without adhering to the wall, but sticking close to it, and "bulldoze" their way through to destroy newly forming and already formed platelet clots.

Of course, this explanation is highly hypothetical, and there are many other possibilities to be tested.

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Ulcerostatic Effect of *Bacillus mucilaginosus* Exopolysaccharide and its Possible Mechanisms

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The search for and detailed study of stimulators of repair processes in connective tissue are under special investigation at present. As has been shown, during gastric ulcer formation the level of polysaccharide components in the connective tissue of the stomach drops [2]. It may thus be expected that the administration of certain polysaccharides to the organism will produce an ulcerostatic effect. In this respect, investigation of exopolysaccharides from *Bacillus mucilaginosus*, which possess a biostimulating activity, seems rather promising [7,10]. Here, we report on a study of the ulcerostatic effect of *Bacillus mucilaginosus* polysaccharide.

MATERIALS AND METHODS

The experiments were performed on nonpedigree albino rats weighing 150-180 g. Gastric ulcer was induced by the acetate me- thod [9]. Polysaccharide from extracellular mucus of *Bacillus mucilaginosus* was isolated by precipitation, separation from ballast material and freeze-drying. Starting from the first day of the experiment, the animals received intraperitoneal injections of an aqueous solution of the polysaccharide (PS) in a dose of 5 mg/kg/day. The animals were divided into groups of 10 rats and were decapitated on the 7th, 14th, and 28th day. The stomach was removed and the area of lesion was measured. The ulcerated regions were subsequently excised and immersed in liquid nitrogen prior to monitoring the development of the connective tissue components.

The quantitative method was employed to study the glycopro- tein exchange in each group of aminals: the lesion tissue was dehydrated in acetone and delipidated subsequently in an ethanol- chloroform mixture and ethanol. The samples were homogenized and hydrolyzed. The total concentration of hexoses and hexuronic acids was determined in the first neutralized hydrolysate (0.5 n HCl, 100°C, 15 min). One of the homogenized samples was used for fractionation of glucosaminoglycans (GAG). Another sample was used for determination of sialic acids [5, 6, 8]. The effect of PS was compared to that of methyluracil (MU), which stimulates cell regeneration and lesion healing [1].

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