BLOOD AND LYMPH IN EXPERIMENTAL MYOCARDIAL ISCHEMIA AND ARTERIAL HYPERTENSION

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UDC 616.127-005.4+616.12-008.331.1]-092.9-07:616.155+612.423

KEY WORDS: experimental myocardial ischemia, arterial hypertension, lymph, specific electrical conductance.

One component of the pathogenesis of hypertension and myocardial infarction is the relationship between polymorphonuclear leukocytes and leukotrienes, discovered by a number of research workers [9-13]. Polymorphs, which produce leukotrienes, are involved in the regulation of the immune system, by their action of T lymphocytes. These mediators (leukotriene B4) have been shown to possess a powerful vasoconstrictor action, including on the coronary vessels [10, 11, 13]. Animals with a low leukocyte count or which have received a course of treatment with 5-lipoxygenase inhibitors in experimental myocardial infarction have significantly less myocardial damage than the corresponding control group of animals [11, 12].

In the investigation described below the cell composition of the blood and lymph was compared in animals with inherited stress-induced arterial hypertension (ISIAH) and with experimentally induced myocardial infarction.

METHODS

Altogether 60 male Wistar and ISIAH rats weighing 160-200 g and aged 2.5-3 months were used. The animals were divided into 5 groups: group 1 (10 rats) was the control, group 2 consisted of hypertensive ISIAH rats, and group 3, 4, and 5 consisted of Wistar rats in which a model of myocardial infarction was created under ether anesthesia [3, 6]. The peripheral blood and central lymph, obtained from the cisterna chyli were studied, because of their high informativeness and easy access under both experimental and clinical conditions. Films were prepared by the standard method and stained by the May—Gruenwald and Romanovsky—Giemsa methods [8]. Central lymph was collected under ether anesthesia with the aid of a glass micropipet from the ciserna chyli of the thoracic duct. The wall of the duct was pierced by the point of a micropipet and fixed [4]. Lymph, in a volume of 0.6-0.8 ml, was collected with a rubber tube and syringe, connected to the micropipet. To prevent the lymph from clotting heparin was added to the tube in a dose of 500 U/ml. The lymph was then centrifuged for 10 min at 1500 rpm. Films were prepared from the residue thus obtained. Blood and lymph cells were counted by the usual method under magnification of 700 [7]. Specific electrical conductance of the blood and lymph was investigated with the aid of a special laboratory apparatus, consisting of four parts: a GZ-112 low-frequency generator, a V3-57 millivoltmeter, a switch, and a conductometric cell [2]. For filling the conductometric cell also was collected from the cisterna chyli of the thoracic duct, blood of 0.8-1.0 ml was obtained from the rat's inferior vena cava. Depending on the relative changes

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Laboratory for Problems in Ontogeny, Research Laboratory of Clinical and Experimental Lymphology. Laboratory of Genetic Bases of Neuroendocrine Regulation, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 4, pp. 349-352, April, 1992. Original article submitted May 20, 1991.

0007-4888/92/0004-0453$12.50

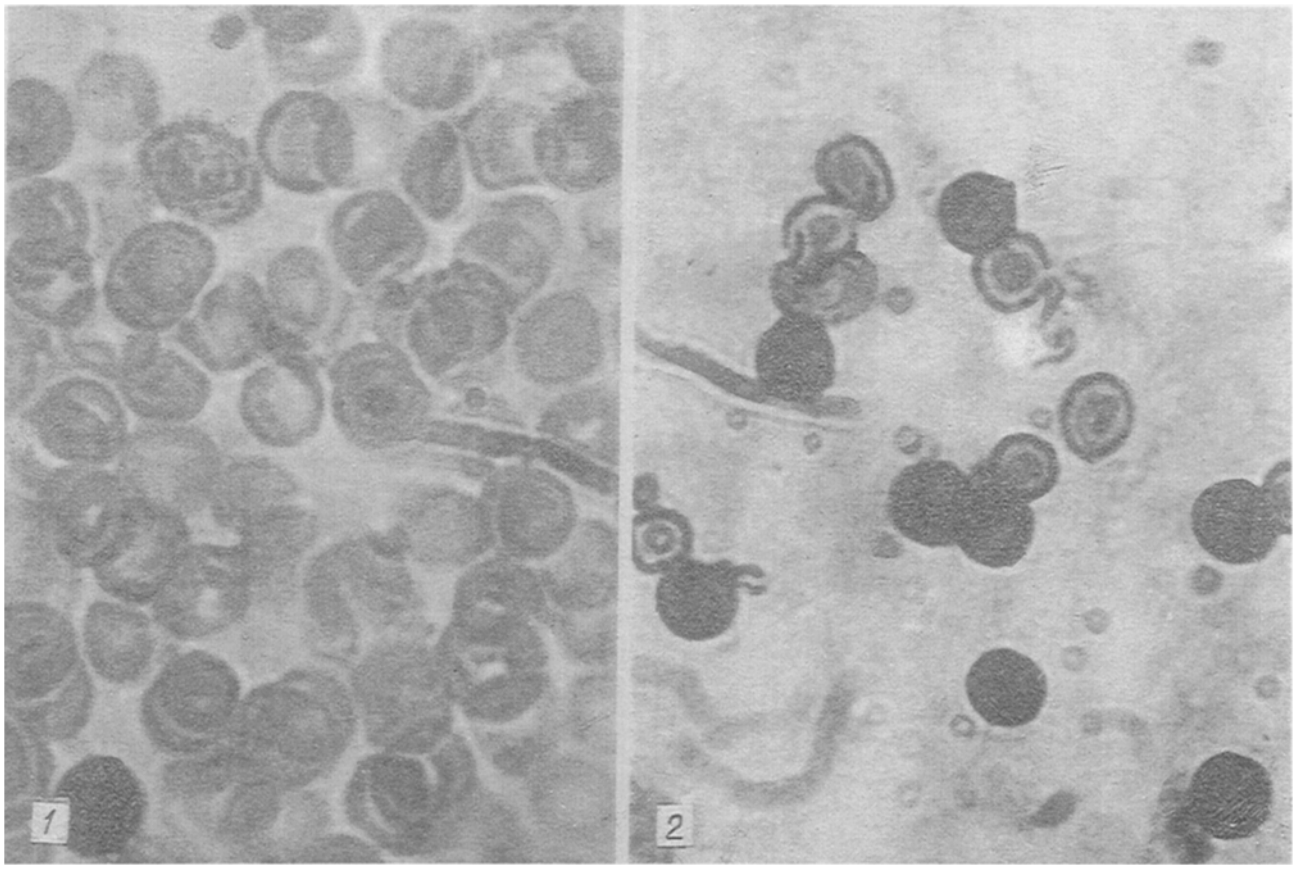

Fig. 1 Fig. 2

Fig. 1. Rouleaux formation by erythrocytes in peripheral blood of ISIAH rats. 900×. May—Gruenwald stain.
Fig. 2. Small lymphocytes and erythrocytes in central lymph of ISIAH rats. 900×. May—Gruenwald stain.

in specific electrical conductance, the quantitative and qualitative composition of the blood and lymph was determined [5]. Investigations in groups with experimental myocardial ischemia were carried out under ether anesthesia on the 1st, 3rd, and 7th days after the operation. Lymphograms and leukocyte formulas were produced for all groups of animals on the basis of the results of counts of the blood and lymph films. The results of the study of specific electrical conductance were compared with an alternating current with a frequency of 1000 Hz. The results were subjected to statistical analysis by Student's test.

RESULTS

All the data obtained during the investigation were compared with the control group (Table 1). The number of basophils and monocytes was significantly increased in the leukocytic formula of animals of the hypertensive strain, the total number of neutrophils was within the limits of the control value, but there was a shift to the left on account of juvenile and segmented forms. Agglutination of erythrocytes into "rouleaux" was observed in the blood (Fig. 1). In the lymphograms of these groups of animals the percentage of lymphoblasts, small lymphocytes (Fig. 2), monocytes, and mature plasma cells was significantly increased, whereas the fraction of medium-sized lymphocytes was reduced (Table 1). Specific electrical conductance of the blood and lymph of the animals with arterial hypertension was significantly raised compared with the control values (Fig. 3). Taking the results into consideration it can be postulated that these differences were due to a change in the electrical properties of the blood and lymph cell membranes [5].

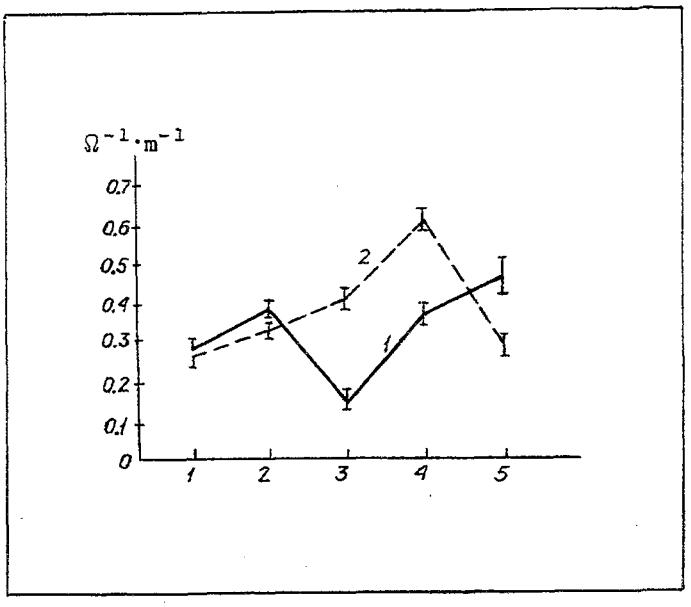

Fig. 3. Specific electrical conductance (in $\Omega^{-1} \cdot m^{-1}$) of blood (1) and lymph (2). Abscissa) group of animals.

TABLE 1. Peripheral Blood Leukocyte Formula and Central Lymph Lymphogram of Rats with Arterial Hypertension and at Different Stages of Experimental Myocardial Ischemia (%) (M ± Sm)

Test object	Cell composition	Control	ISIAH	Time after experimental myocardial ischemia (days)		
				1	3	7
Peripheral blood	Basophils	0	0,64±0,260*	0,125±0,081*	0,75±0,211*	1,5±0,408*
	Eosinophils	2,0±0,95	3,5±0,866	3,625±0,099*	1,0±0,113	1,8±0,402
	Neutrophils	18,5±1,518	21,41±1,282*	25,25±0,433*	21,12±1,860	14,3±0,982*
	—Juvenile	0,5±0,320	2,28±0,434*	6,76±0,510*	2,25±0,211*	2,2±0,374*
	—Stab cells	1,25±0,392	9,28±1,252*	7,37±0,919*	8,75±1,040*	6,3±0,501*
	—Segmented	16,75±2,210	9,85±0,863*	11,12±0,430*	10,12±0,506*	5,8±0,416*
	Lymphocytes:	65,2±1,821	65,27±5,621	67,37±2,014	76,62±3,252*	71,45±2,973*
	Small	42,2±1,322	39,85±3,794	45,0±0,947*	52,62±1,249*	38,3±0,402*
	Medium sized	23,0±1,928	25,42±1,355	22,37±0,505	24,0±0,790	33,15±1,368*
	Monocytes	3,6±0,821	9,28±0,770*	7,75±0,818*	4,62±0,245	11,3±0,343*
Lymphoblasts	Lymphoblasts	0	0,62±0,263*	0,4±0,163*	0,4±0,163*	1,0±0,298*
	Lymphocytes	93,24±3,492	89,37±2,826*	87,6±2,148*	92,4±2,253	89,0±2,128*
	—Small	67,62±2,162	72,37±1,751*	74,2±1,103*	54,0±1,801*	40,2±1,549*
	—Medium sized	25,62±1,860	17,0±1,435*	13,4±1,941*	38,4±2,450*	48,8±0,489*
	Plasma cells	1,32±0,242	1,65±0,378	0,4±0,163*	1,59±0,228	1,6±0,182
	—Mature	0,57±0,297	1,25±0,365*	0,4±0,163	1,09±0,368	1,2±0,249*
	Immature	0,75±0,250	0,4±0,163	0	0,5±0,166	0,4±0,162
	Monocytes	5,5±0,500	7,5±1,180*	11,5±0,453*	5,6±0,541	8,0±0,894*

*Values differing significantly from control ($p < 0.05$).

In group 3, one day after experimental myocardial ischemia, the number of basophils and monocytes in the blood was increased, and the shift to the left of the leukocytic formula persisted due to juvenile forms of neutrophils. The number of small lymphocytes was increased. In the lymph at this stage the number of lymphoblasts, small lymhocytes, and monocytes was increased, whereas the number of medium-sized lymphocytes and immature plasma cells was reduced (Table 1). Specific electrical conductance of the blood fell sharply, possibly due to an increase in the number

of small lymphocytes and of erythrocytes with altered configuration, and also to a shift in the biochemical composition of the blood [1]. The electrical conductance of the lymph was increased compared with the control (Fig. 2).

The number of basophils and of small and medium-sized lymphocytes in the blood was increased 3 days after experimental myocardial infarction compared with the previous stage. The percentage of juvenile neutrophils and of monocytes was reduced. In the lymph there were fewer small lymphocytes but an increase in the number of lymphoblasts and medium-sized lymphocytes (Table 1). Specific electrical conductance on the 3rd day after infarction was increased in both blood and lymph. In the blood, this was evidently the result of a decrease in the number of juvenile neutrophils and monocytes, and of all the modified erythrocytes, whereas in the lymph it was due to a decrease in the fraction of small lymphocytes and monocytes compared with the previous experimental groups, and an increase in the number of medium-sized lymphocytes, in which qualitative changes were possibly present in the cell membranes (Fig. 3).

A further increase in the number of basophils, medium-sized lymphocytes, and monocytes in the blood was found 7 days after myocardial infarction compared with the previous experimental group, whereas the percentage of small lymphocytes, medium-sized lymphocytes, mature plasma cells, and monocytes in the lymph was increased (Table 1). The specific electrical conductance of the blood was increased. This was evidently connected with a change in its quantitative composition, expressed as a decrease in the number of neutrophils and small lymphocytes and an increase in the percentage of basophils, monocytes, and medium-sized lymphocytes, whose membranes probably acquire new and different properties. The electrical conductance of the lymph decreased, and approached the control values. This tendency was perhaps due to the increase in the number of cells in the lymph and restoration of the properties of their membranes (Fig. 3).

Features of similarity in the quantitative and qualitative changes in the blood and lymph associated with cardiovascular pathology are deduced from these observations. Basophilia, eosinophilia, and monocytosis characteristics of hypertensive rats. The shapes and sizes of the erythrocytes also differ in hypertensive rats from those in the control, but the differences are more marked in animals with experimental myocardial infarction. In the lymph of ISIAH rats compared with the control group, lymphoblasts appeared, the number of small lymphocytes and monocytes increased, but the population of medium-sized lymphocytes decreased. Experimental myocardial ischemia was characterized by a gradual decrease in the number of small lymphocytes and monocytes in the lymph, and an increase in the fraction of lymphoblasts, medium-sized lymphocytes, and mature and immature plasma cells in the lymph. The results of measurements of electrical conductance of the lymph and blood agree completely with the time course of changes in their qualitative and quantitative composition. The results of the investigation suggest that in rats with arterial hypertension and experimental myocardial ischemia the leukocytic formula shifts toward an increase in juvenile forms of granulocytes.

Comparison of the leukocytic formulas and lymphograms of the hypertensive rats and of animals with experimental myocardial ischemia led to the following conclusions. If the cell composition of the blood and lymph is compared, similarity can be found in the hypertensive rats and animals with experimental myocardial ischemia lasting 3 days, and in the case of the lymphogram, in the 1st day after ischemia. A unique "crossover" is observed if these curves are superposed, and common "points" are found with similar quantitative cell composition. For the leukocytic formula these points are all forms of neutrophils, for the lymphogram they are the medium-sized lymphocytes. The relative percentages of the other cells did not change significantly, differing from the control normotensive group in the unidirectional character of their reactions.

The results suggest that the development of acute myocardial ischemia in hypertensives can be predicted by studying the cell composition of the lymph and blood. Results obtained by the study of electrical conductance confirm this hypothesis. The earlier coincidence of the number of cells in the lymph of animals with experimental myocardial ischemia and with arterial hypertension than in the blood points to rapidly developing changes in the lymphatic system, and, consequently, it is a more sensitive method of diagnosis of an impending critical period in the course of arterial hypertension.

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