PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF PERIPHERAL BLOOD LYMPHOCYTES IN ISCHEMIC HEART DISEASE

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The development of atherosclerosis and of its clinical manifestation, namely ischemic heart disease (IHD), is accompanied by changes in lymphocyte function. Insufficiency of the T-immune system, for example, has been observed during the study of blast transformation under the influence of phytohemagglutinin [1]. On the other hand, great importance is attached to possible specific sensitization of lymphocytes, significantly altering the properties of the cell surface [3]. However, the causes leading to such disturbances remain largely unexplained.

In the investigation described below the spin probes method was used to study the physical properties and concanavalin A- (con A-) initiated structural changes in the membranes of peripheral blood lymphocytes from normal individuals and patients with IHD. To discover the possible causes of structural modification of the membranes, the relative cholesterol content in the cells was determined. The functional capacity of the lymphocytes was judged by testing mitogen-stimulated redistribution of endogenous Ca^{++} and the reaction of cap formation in response to injection of rabbit antiserum, labeled with fluorescein isothiocyanate (FITC).

EXPERIMENTAL METHOD

Peripheral blood lymphocytes were isolated by the method in [4] in a Ficoll-Verografin density gradient, followed by washing 3 times with medium 199 (pH 7.2). The test suspension contained $5 \cdot 10^7$ cells in 1 ml with a viability of more than 96%, in the trypan blue test.

Concentrations of cholesterol and phospholipids were determined after extraction of the lipids by Folch's method as described previously [2]. On the basis of the results the cholesterol/phospholipids (Ch/PL) ratio was calculated, numerically equal to the ratio between quantities of cholesterol and phospholipids in moles.

A stearic acid derivative with nitroxyl fragment in position 5 relative to the carboxyl group, 5-doxyl stearate (5-DS, from "Sigma," USA) was used as the spin probe. The method of introduction of the probe, of recording the EPR spectra, and calculating the parameter of order was described previously [2].

The functional properties of the lymphocytes were studied by immunofluorescence [5], using FITC-labeled rabbit antiserum to human immunoglobulins (obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology). The sum contained 0.02% sodium azide to prevent the reaction of cap formation during staining.

The redistribution of Ca^{++} during activation of the cells by con A was studied by measuring fluorescence of chlortetracycline (CTC) by the method described in [8]. Fluorescence was recorded on a "Hitachi MPF-4" spectrofluorometer at room temperature.

EXPERIMENTAL RESULTS

Investigation of peripheral blood lymphocytes from patients with IHD and normal subjects revealed significant differences in the structural organization and chemical composition of their cytoplasmic membranes. The parameter of orderliness of 5-DS and, consequently, orderliness of the fatty-acid chains of the lymphocyte membrane phospholipids, were higher in patients with IHD (Table 1). Meanwhile an increase was observed in the cholesterol content of the mem-

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TABLE 1. Physicochemical Properties of Lymphocytes from Patients with IHD and Normal Blood Donors (M ± m)

Group of subjects studied	S_0, relative units	ΔS, relative units	Ch/PL, moles/mole
Control (18)	0,602±0,007	0.037±0.005	0,57±0,04
Patients with IHD (25)	0,622±0,007*	0,020±0.004	0,62±0,02**

Legend. Here and in Table 2: *p < 0.05, **p < 0.001 compared with control. Number of tests shown in parentheses.

TABLE 2. Results of Cap-Formation Test with Polyvalent Anti-Immunoglobulin Serum, Labeled with FITC (M ± m)

Group of subjects studied	Number (in %) of cells with caps relative to total number of fluorescent cells			
	0 min	30 min	60 min	90 min
Control (10)	12±2	54±4	46±3	30±2
Patients with IHD (15)	26±5*	43±3*	55±5**	50±4*

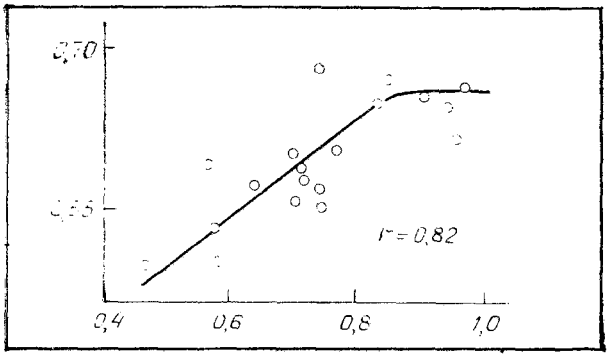

Fig. 1. Dependence of orderliness of the 5-DS probe on relative cholesterol content (Ch/PL) in human lymphocytes. Ordinate, S (in relative units); abscissa, ratio Ch/PL (in moles/mole).

branes of their lymphocytes, as shown by an increase in the Ch/PL ratio. The increase in orderliness of the lymphocyte membranes was evidently linked with cholesterol accumulation. To confirm this conclusion, dependence of the parameter S on the molar fraction of cholesterol was studied. With an increase in the Ch/PL ratio, an increase was observed in the parameter of orderliness, reflecting an increase in viscosity of the lipid phase of the membranes (Fig. 1). In other words, changes in structure of the plasma membranes of the peripheral blood lymphocytes of patients with IHD are largely associated with cholesterol accumulation in the cell.

To determine more precisely the role of the physical state of the membranes in the activation reactions, we studied structural transformations taking place in the microenvironment of the probe during the first minutes of interaction of con A with lymphocytes. Activation of lymphocytes with con A led to reduction of the orderliness of the probe, taking place as a result of reduction of the packing density of the fatty acid chains of the phospholipids in its microenvironment. This process is due to the formation of lipid domains during aggregation and redistribution of con A receptors on the surface of the lymphocytes, and the subsequent process of cap formation [6]. The difference (ΔS) between the parameters of orderliness, measured before (S_0) and 10 min after (S_{10}) mixing of con A with the lymphocyte suspension, was significantly less in patients with IHD.

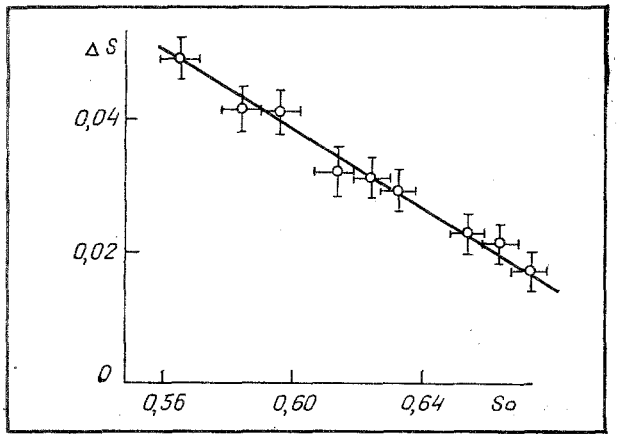

Fig. 2

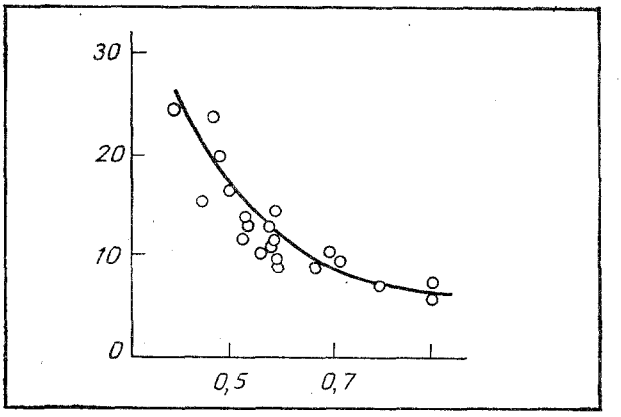

Fig. 3

Fig. 2. Dependence of changes in orderliness of 5-DS in human lymphocytes 10 min after addition of con A on its initial value in intact cells. Medium 199 (pH 7.2), 10^8 cells/ml; con A 10^{-4} mg/ml, 37°C.

Fig. 3. Dependence of reduction of intensity of fluorescence of CTC in human lymphocytes 1 min after addition of con A on molar fraction of cholesterol in cells. Conditions and legend as to Fig. 3. Abscissa, Ch/PL ratio (in moles/mole).

Dependence of the changes in mobility of the probe after interaction between lymphocytes and mitogen on the initial value of the orderliness parameter in intact cells is shown in Fig. 2. Clearly with an increase in S_0, the value of ΔS decreases. This means that an increase in the degree of orderliness of phospholipids of the lymphocyte membranes in patients with IHD is accompanied by inhibition of structural transformations induced by the mitogen. In turn, this may give rise to a slower course of the reaction of cap formation. The results of this test are given in Table 2. Differences were found in the distribution and displacement of the immunoglobulins over the cell surface. Characteristically, in all preparations immediately after mixing with the FITC-labeled polyvalent anti-immunoglobulin serum, the percentage of cells carrying receptor caps, i.e., the number of initially activated lymphocytes, was greater in patients with IHD. Conversely, the increase in the number of these cells among the total number of fluorescent cells after incubation for 30 and 60 min was much smaller in these tests. It must be pointed out that the largest number of lymphocytes forming caps on their surfaces was the same in preparations from patients with IHD and from normal individuals, and was about 55% of the total number of fluorescent cells, i.e., of cells capable of binding with the serum. The differences obtained are thus evidence of a decrease in the rate of cap formation, but the number of cells undergoing such changes in response to the action of antigen remained the same in IHD as in the normal subject.

If these data are compared with the results of investigation of the structural organization of the lymphocyte membranes, it can be concluded that the low velocity of the cap formation reaction in patients with IHD can evidently be explained by immobilization of the immunoglobulins as a result of an increase in density of the lipid matrix of the membranes. Changes in structure of the membranes may become the cause of inhibition of the primary stages of activation, directly linked with the plasma membrane. These stages, in particular, include the process of redistribution of endogenous Ca^{++} – an important regulator of the mitogenic response. The outflow of Ca^{++} from the inner phospholipid layer of the plasma membrane into the cytoplasm is known to be the trigger signal for a cascade of reactions, ending with proliferation [7, 9]. There is every reason to suppose that the changes in the structural organization of the lymphocyte membranes found in patients with IHD involve differences in the mitogen-induced redistribution of endogenous Ca^{++}.

The decrease in the intensity of fluorescence of CTC in the cells after their activation by con A, which is linked with displacement of Ca^{++} from the hydrophobic environment of the membrane into the polar (cytoplasm) in fact takes place significantly faster in the immunocytes of healthy blood donors. These differences are increased in intensity with an increase in the relative cholesterol content in the cells. In other words, accumulation of cholesterol and the increase in viscosity of the lipid phase of the membranes related to it, leads to a decrease in the intensity of mitogen-stimulated Ca^{++} redistribution (Fig. 3). It

can thus be concluded from these investigations that peripheral blood lymphocytes of patients with IHD possess more viscous membranes, whose orderliness increases proportionally to the increase in the cholesterol content in the cells. An increase in viscosity of the membranes is one of the more important causes of depression of the function of these cells in IHD, and disturbances of the redistribution of Ca^{++} ions are evidently one mechanism realizing the effect of modification of membrane structure on cell function.

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ANTIBODY FORMATION TO AUTOLOGOUS ERYTHROCYTES AFTER IMMUNIZATION OF NORMAL MICE AND OF MICE TOLERANT TO THE IMMUNIZING ANTIGEN, WITH RAT ERYTHROCYTES

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A method of inducing an autoimmune response to erythrocytic antigens in mice by repeated injection of a cross-reacting antigen, namely rat erythrocytes (RE), was developed in 1973 [10]. For a long time (over 3 months) antibodies to autologous erythrocytes were found in animals immunized in this way. Several parameters of the response to autologous erythrocytes have been investigated in the USSR [1, 2] and in other countries [8, 9], but the conditions of formation of this autoimmune response have not been adequately studied.

In the investigation described below this problem was investigated by comparing the effectiveness of induction of the autoimmune response in mice capable of synthesizing antibodies to RE, and in animals specifically areactive to that antigen.

EXPERIMENTAL METHOD

Male CBA/CaLacSto and BALB/B/c mice and (CBA × C57BL/6)F_1 hybrids, and August rats were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR; male CC57BR/Mv mice were obtained from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. For immunization, 2×10^8 RE were injected intraperitoneally 4-5 times with intervals of 7-10 days between injections.

The overall antibody titer to RE in the serum was determined by the hemagglutination test on the 7th-9th day after the last immunization. Antibodies of the IgG class were determined after preliminary incubation of 50 µl of test serum in a dilution of 1:5 with 50 µl of a 1% solution of mercaptoethanol ("Serva") in buffered physiological saline (pH 7.4). The

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