EXPRESSION OF SUPERFICIAL MARKERS ON PERIPHERAL BLOOD LIMPHOCYTES IN IHD PATIENTS IN THE CONDITIONS OF YAKUTIA.

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Abstract

A research objective was an assessment of an expression of superficial markers of lymphocytes in patients with IHD of a various form depending on ethnicity. The received results of the comparative analysis depending on IHD form allow to confirm that destabilization of IHD is characterized by increase of an expression of CD19 + (B-lymphocytes), activation markers of CD25 + (a receptor to IL-2) and CD71 + (a trancferrine receptor), and also molecules of intercellular adhesion of CD54 + (ICAM-1). At unstable angina in persons of native nationality signs of deficiency of the T-cellular link (T-lymphocytes, T-helper), and also significant increase of expression CD11b + (integrine) in comparison with persons of non-native nationality are revealed.

Keywords: atherosclerosis, stenocardia, immune system, activation antigens.

Introduction.

According to modern views, the atherosclerosis is considered as a number of consistently developing cellular and molecular infringements which can be described as chronic inflammatory disease [4]. The quantity increase monocyte - and granulocyte - trombocyting complexes, observed at patients with atherosclerosis, according to researchers, testifies to activation of inflammatory process in the damaged vein. At the development of cardiac infarction and an unstable stenocardia the intracoronary thrombosis developing on a place of rupture, damage of an atherosclerotic plaque or endothelium erosion [2]. A number of authors considers leukocyte - trombocyte aggregation and aggregation of leucocytes as a possible key link of development of sharp coronary syndromes [14]. Along with distinct spontaneous aggregation of trombocyte in vivo the literary data testifies about various changes and decrease aggregation of trombocytes blood plasma in vitro at patients with cardiac infarction during the sharp period [15]. Leukocyte – trombocyt adhesions on extracellular matrix and intercellular aggregation act as a ligand of molecula ICAM-1: αIIb / β3 and β1-bound интегрины, P-selektin - PSGL (P-selectin glycoprotein a ligand-1) and CD40 - CD40L [6].

At sharp coronary syndromes there is an activation of trombocytes, monocytes and granulocytes of peripheral blood. The quantity increase monocyte - and granulocyte - trombocyte the complexes, observed at patients with an atherosclerosis, according to many researchers, testifies to activation of inflammatory process in the amazed vessel [16, 9].

Participation T-lymphocyte in atherogenes is caused by their role in an antigen recognition, clone expansion, initiate the cellular-mediated inflammatory answer and is confirmed by results immunological the researches showing an associatively of demonstration of clinical symptoms of an atherosclerosis and processes T-cellular activation [10]. It is shown the chronic T-cellular activation accompanied by increase of quantity lymphocyte which expressed activate antigens, it is observed not only in a damage zone, but also in peripheral blood and meets at patients various forms of an atherosclerosis [3, 12].

Believe that activation of T- lymphocyte and, most likely, CD4 +-T- helpers occurs in the T-zone of lymph nodes, whence the activated T-cells migrate in corresponding sites of a vascular wall [5]. The estimation of the immune status patients of atherosclerosis has allowed establishing at them essential disbalance immunological parameters, characterized by high activity humoral immunity interfaced to relative deficiency T-cellular link of immune system [1]. Quantitative changes circulating lymphocytes, expressing activating antigens can be reflexion activating processes in a zone of damage of a vessel

Interaction of blood cells with a vascular wall is the major component of immune protection of an organism, providing constant transvascular migration lymphocytes in tissue and lymph nodes with view of detection of alien antigens. Besides it, the expression in the conditions of damage of fabrics adhesion molecules on endothelium and inflammatory blood cells is to their initial stages recruiting in the centre of an inflammation and initiates a wide spectrum of changes, in the basic, protective character, but resulting at excessively long and intensive activation to additional development dystrophic and necrotic changes. [7]. Thereby, considering small number and discrepancy of the data about a functional condition lymphocytes at atherosclerosis, and also existing ethnic distinctions on growth of disease and death rate from cardiovascular diseases of native population at smaller expressiveness of an atherosclerosis of coronary arteries, than at nonnative Yakutia, considerable interest is represented by an estimation of an expression of superficial markers lymphocytes to peripheral blood at patients ischemic heart disease (IHD) of the various form (a stable and unstable stenocardia) depending on an ethnic accessory.

Materials and methods. Into research have entered 71 sick IHD (all men, middle age 54,6 ± 7,1 years). All patients have divided on two groups depending on form IHD: 1 group included 32 patients with the diagnosis stable stenocardia (middle age 53,75 ± 1,29 years); into 2 group have entered - 38 patients ИБС with an unstable stenocardia (branch of resuscitation and intensive therapy), middle age 58,56 ± 1,61 years. Subsequently these groups have separately been divided depending on an ethnic accessory: in 1 group of patients with a stable stenocardia of the person of

native (Yakuts) have made 13 men, nonnative - 19 men. In group of patients with an unstable stenocardia of the man of a native have made 22, nonnative - 16 persons.

For immunological researches used lymphocytes, allocated of peripheral blood of patients. Blood took away on an equal footing: in the morning, on an empty stomach, in volume 5 - 7 ml. Immunofenothyping of lymphocytes spent a method flowing cytometrya (FACSCalibur, Becton Dickinson) with use monoclonalic antibodies with a threefold label: CD3FITC + CD4RPE + CD45RPE-Cy5; CD3FITC + CD19-RPE + CD45RPE-Cy5; CD3FITC + CD8RPE + CD45RPE-Cy5; CD16FITC + CD19RPE + CD3RPE-Cy5; with one label CD25-RPE; CD11b-RPE; CD71-FITC; (Dako); CD54-RPE, CD95-RPE (Becton Dickinson), CD62L-FITC (Sorbent, St.-Petersburg). Relative maintenances of lymphocytes, expressive following markers are analysed: CD3 + - T-lymphocytes; CD4 + - T-helpers; CD8 + - cytotoxic T-lymphocytes; CD16 + - NK - killers; CD19 + - B - lymphocytes; CD11b + - αм - chain integrin; CD25 + - α - chain of receptor ИЛ-2; CD54 + - adhesion receptor ICAM - 1; CD62L + - adhesion molecule L-selectine; CD71 + - receptor transferrine; CD95 + - proapoptosis marker.

Results and discussion. The carried out analysis of the indicators characterizing structure of the basic subpopulations of peripheral blood of patients IHD, has revealed at sick of an unstable stenocardia significant increase of relative and absolute maintenance CD19 + in comparison sick of a stable stenocardia (tab. 1). Thus the greatest average value of relative and absolute maintenance CD19 + is marked at persons of native without significant distinctions (tab. 2). Comparison of parameters of the T-cellular link of immunity depending on forms IHD has no significant distinctions, and average values are in limits reference sizes. However, at comparison of indicators sick of an unstable stenocardia depending on an ethnic accessory has revealed that at native men signs of T-cellular deficiency, i.e. decrease in relative and absolute maintenance CD3 + and CD4 + (tab. 2) are observed. An estimation immunoregulate index (IRI), i.e. parity of number CD4 + and CD8 + (the norm 1,6 - 2,2) depending on form IHD has shown a tendency of distinctions (p=0,06). The least average value IRI is observed at persons of a radical nationality at an unstable stenocardia at the expense of expressed decrease CD4 + (T-helpers).

For an estimation of ability of cages of immune system to activation by us it is spent fenotyping "early" CD25 +, CD71 + and "late" CD95 + activation markers. CD25 + - receptors to IL-2 expressed mainly on activated T-helpers. The increase in their number arises at proliferation T-cells under the influence of T-cellular ростового growthing factor IL-2, and maintenance CD25 + positive cells above 15 % testifies to activation of immune system. Comparison of groups depending on form IHD has shown significant increase relative (p=0,006) and the absolute

maintenance (p=0,01) CD25 + at sick of an unstable stenocardia, than at sick of a stable stenocardia. Comparison depending on an ethnic accessory has not revealed significant distinctions, however in group sick the unstable stenocardia marks a tendency to increase of relative and absolute maintenance CD25 + positive cages (p=0,09) at persons of not radical nationality.

CD71 + (receptor of trancferrine) is early activation marker of lymphocytes appears on leukocytes at their activation. It is found out on the majority of sharing cells. Increase of number CD71 + lymphocytes with high proliferate activity reflects processes of activation of immune system, occurrence of early predecessors, infringement of normal process of maturing immunocytes. The relative and absolute maintenance of lymphocytes with high proliferate activity, expressing CD71 +, at an unstable stenocardia has appeared significantly above, than at a stable stenocardia (p=0,042; p=0,036, accordingly). At comparison of groups depending on ethnic accessory significant distinctions are revealed at a stable stenocardia: at nonnative relative and absolute maintenance CD71 + has appeared above, than at native though values are in limits of referential sizes.

It is necessary to underline that there are fundamental distinctions between consequences apoptose of endothelium and inflammatory cells in a zone of damage of a vessel. CD95 + (Fas, APO-1) concerns family of receptors of the factor necrosis to a tumour/factor of growth of nerves, it is presented mainly on T-cells, in particular, on T-helper. The increase in number of cages with CD95 + - the receptors perceiving a signal of an induction apoptosis, can reflect as activation of immune system (CD95 + - a marker of "late" activation), well a readiness marker to apoptose. In our research average values of relative maintenance CD95 + in all investigated groups exceed referential sizes, and the greatest value is marked at sick of an unstable stenocardia. Statistically significant distinction on an ethnic accessory is established in group sick of an unstable stenocardia: at nonnative absolute quantity CD95 + significantly above, than at radical (p=0,04) though values is in limits of referential sizes. It is necessary to notice that strengthening apoptose of lymphocytes with participation CD95 + is connected with processes of nonspecific and defective activation immune cells, and also infringement of processes of their normal maturing. Thus, the results received by us according to ability of cages of immune system to activation testify that an expression «early and late» activate markers at an unstable stenocardia are raised, than at a stable stenocardia and are most expressed at persons of not radical nationality.

The expression adhesion molecules on endothelium and leukocytes is their initial stage recruitment in the inflammation centre that is accompanied by occurrence of a wide spectrum of the reactions having in the basis protective character, but resulting at excessive duration or intensity to

additional development dystrophic and necrosis changes. Such process is characteristic for a local inflammation in a wall of vessels which underlies atherosclerosis development. We define levels of an expression such adhesion molecules as CD54 + (ICAM-1) - a molecule of intercellular adhesion of 1 type, belonging to superfamily of antibodies; CD11b + - αм - a chain integrin CR3; and CD62L + - L - selectine.

The comparative analysis depending on form IHD has established significant increase at sick of an unstable stenocardia relative (p=0,041) and absolute (p=0,01) quantities CD54 + positive lymphocytes, than at sick of a stable stenocardia. It is necessary to notice that the expression of these molecules on endothelium vessels has constant character, but considerably amplifies at its stimulation proinflammatory cytokines and modified lipoproteins. On based leukocytes of peripheral blood CD54 + expressed it is weak, amplifies at activation T- lymphocytes, V-lymphocytes and monocytes and promotes their mutual adhesion with formation of large multicellular units. An expression as ICAM-1 and VCAM-1 according to authors [13] it was not marked in the intact arteries, it was found out only at activation endothelium proinflammatory медиаторами FNO - α, IL-1, interferon and was dependent on factor NF-kB.

CD11b + - αм the chain in a combination with β - a chain (CD18 +) forms specific to leukocytes integrin, designated as Mas-1 (macrophag receptor-1) or a receptor of inactivating complement CR3, expressed on myeloid cells and natural killers. We establish significant increase (p=0,041) relative maintenance CD11b + at a stable stenocardia, in comparison with sick of an unstable stenocardia. The comparative analysis depending on an ethnic accessory has revealed significantly high (p=0,000) indicators CD11b + at persons of a radical nationality sick of an unstable stenocardia.

CD62L + - L- selektin it is constant expressed on a tip pseudopodia mononuclear and provides an attachment lymphocyte to endothelium cell. At activation lymphocytes often there is "washing off" L - selectine that is accompanied by activation b2 - integrin complex (CD11/CD18) of lymphocytes and monocytes [11]. Loss of adhesive molecules from a surface of cages can be one of forms of negative regulation of an inflammation. In our research relative maintenance CD62L + at sick of an unstable stenocardia significantly more low (p=0,001), than at patients with a stable stenocardia, i.e. infringements of regulation L-selektin of the mediated adhesion at patients with an ustable stenocardia are most expressed. Thus, the estimation of molecules of adhesion at patients IHD has shown ambiguous changes on character. So at an unstable stenocardia expression CD54 + is raised and infringements L - selektine are expressed, to the mediated adhesion (decrease CD62L +). At a stable stenocardia the raised expression CD11b +. Ethnic distinctions are revealed in group

sick of an unstable stenocardia: at native maintenance CD11b + is significantly raised, than at persons of nonnative.

Such, in the image, the received results of the comparative analysis depending on form IHD allow to confirm, destabilization IHD is characterised by increase of expression CD19 + (B-lymphocytes), activate markers CD25 + (a receptor to IL-2) and CD71 + (a receptor trancferrine), and also molecules of intercellular adhesion CD54 + (ICAM-1). At an unstable stenocardia at native signs of deficiency of the T-cellular link (T-lymphocyte, T-helper), and also significant increase of expression CD11b + (integrin) in comparison with nonnative.

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Expression of markers on lymphocytes at patients depending on form IHD

Table 1

Parametres		1- stable stenocardia (n=32)	2 - unstable stenocardia (n=38)	p... 1-2
CD3+	%	71,96 ± 1,67	70,52 ± 1,63	
	abs.	1,37± 0,11	1,42 ± 0,11	
CD4+	%	40,72 ± 2,03	40,78 ± 1,55	
	abs.	0,80 ± 0,08	0,82 ± 0,07	
CD8+	%	23,28 ± 1,73	26,65 ± 1,29	
	abs.	0,43 ± 0,05	0,54 ± 0,05	
IRI (CD4+/CD8+)		2,22±0,26	1,69±0,12	
CD19+	%	7,28 ± 1,00	12,15 ± 1,37	*0,006*
	abs.	0,16 ± 0,03	0,23 ± ±0,03	*0,006*
CD16+	%	19,80 ± 1,64	16,50 ± 1,27	
	abs.	0,36 ± 0,04	0,33 ± 0,04	
CD25+	%	16,60 ± 1,89	24,13 ± 1,79	*0,006*
	abs.	0,30 ± 0,04	0,51 ± 0,06	*0,011*
CD54+	%	31,62 ± 3,32	39,97 ± 2,38	*0,041*
	abs.	0,61 ± 0,08	0,81 ± 0,07	*0,01*
CD62L+	%	49,68 ± 2,89	34,13 ± 3,42	*0,001*
	abs.	0,97 ± 0,11	0,67 ± 0,10	
CD95+	%	39,46 ± 3,98	45,45 ± 3,40	
	abs.	0,76 ± 0,11	0,99 ± 0,12	
CD11b+	%	41,25 ± 3,00	33,63 ± 2,17	*0,042*
	abs.	0,74 ± 0,06	0,65 ± 0,06	
CD71+	%	4,56 ± 0,53	10,26 ± 1,37	*0,008*
	abs.	0,10 ± 0,02	0,20 ± 0,09	*0,036*

Expression of markers лимфоцитов at patients ИБС in dependence from an ethnic accessory

Table 2

Parametres		stable stenocardia 1 native n=13	2 nonnative n=19	unstable stenocardia *p... 1-2*	*p... 3-4* 3 native n=22	4 nonnative n=16	
CD3+	%	72,61±2,39	71,52±2,34		67,47±1,95	75,42±1,82	*0,006*
	abs.	1,17±0,11	1,51±0,16		1,18±0,11	1,76±0,18	*0,012*
CD4+	%	37,69±3,09	42,78±2,65		36,86±1,56	45,85±2,42	*0,002*
	abs.	0,62±0,08	0,93±0,11		0,65±0,07	1,06±0,12	*0,004*
CD8+	%	25,84±2,72	21,52±2,21		26,78±1,85	26,92±1,78	
	abs.	0,42±0,05	0,45±0,07		0,45±0,05	0,65±0,09	
IRI (CD4+/CD8+)		1,72±0,26	2,55±0,37		1,57±0,16	1,81±0,15	
CD19+	%	7,07±1,37	7,42±1,42		13,21±2,17	10,35±0,97	
	abs.	0,12±0,03	0,19±0,06		0,22±0,05	0,24±0,04	
CD16+	%	22,92±2,50	17,55±2,08		18,61±1,70	13,92±1,62	
	abs.	0,37±0,06	0,34±0,04		0,34±0,06	0,31±0,04	
CD25+	%	14,66±2,61	17,89±2,65		20,91±2,52	27,40±2,47	
	abs.	0,24±0,04	0,35±0,06		0,41±0,09	0,62±0,08	
CD54+	%	32,69±5,93	30,89±3,99		41,95±3,12	37,35±3,89	
	abs.	0,51±0,11	0,68±0,11		0,73±0,08	0,95±0,15	
CD62L+	%	50,27±4,34	49,29±3,96		34,36±4,38	32,80±5,94	
	abs.	0,84±0,13	1,07±0,17		0,62±0,14	0,71±0,16	
CD95+	%	41,91±5,45	37,62±5,74		44,27±5,61	48,50±4,05	
	abs.	0,64±0,11	0,78±0,08		0,81±0,20	1,17±0,12	*0,04*
CD11b+	%	44,00±4,59	39,27±4,01		40,43±2,44	24,00±2,07	*0,000*
	abs.	0,69±0,08	0,78±0,08		0,71±0,09	0,56±0,07	

CD71+	%	3,28±0,61	5,55±0,66	*0,031*	12,30±1,95	6,93±1,61	
	abs.	0,05±0,01	0,15±0,03	*0,03*	0,21±0,04	0,19±0,44	

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