Typical Changes of Erythrocytes in Chronic Inflammation

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Structural and metabolic status and function of erythrocytes were studied in patients with chronic bronchitis, chronic inflammatory diseases of the upper airways, and chronic colitis. Complex study of the peripheral component of the erythron showed that disorders in erythrocyte morphology and function (decreased erythrocyte dry weight, low concentration of sulfhydryl groups and lipoproteins, decreased content of high molecular weight polypeptides in parallel with increased content of low molecular weight proteins, increased number of transformed cells, and enhanced reversible aggregation) were nonspecific and did not depend on the location of the inflammatory process.

Key Words: *erythrocytes; cytophotometry; electrophoresis; chronic inflammatory diseases*

Inflammation as a typical pathological process underlying the majority of human diseases is a result of reaction of the connective tissue, microcirculation, and blood system to the damaging agent. Inflammation is always associated with pronounced changes in the blood system and its outcome is largely determined by the function of this system, including the function of the erythron [4,6]. Intensive destruction of peripheral blood erythrocytes in patients with chronic inflammatory diseases is determined by changes in cell structure, membrane function, and metabolic disorders. Modern concepts on the development and outcomes of inflammatory reactions in the body are based on the leading role of membrane destructive processes [1,3, 10]. Many aspects of this problem remain little studied, which largely impedes the development of the concept of the pathogenesis of anemias in chronic inflammation.

This study was aimed at detection of the common regularities and mechanisms of disorders of the structural and metabolic status and function of erythrocytes in patients with chronic inflammatory diseases.

MATERIALS AND METHODS

Fifty-three patients with chronic bronchitis (39 men and 14 women), 15 with chronic inflammatory diseases of the upper respiratory tract (6 men and 9 women), and 11 patients with chronic colitis (5 men and 6 women) aged 30-62 years were examined during remission of chronic inflammatory process. Control group consisted of 69 healthy subjects (44 men and 25 women) aged 21-56 years. Venous and peripheral blood was analyzed. No statistically significant differences in the parameters of the peripheral component of the erythron (except dry weight of erythrocytes) were detected between male and female patients with chronic bronchitis and healthy volunteers.

The content of sulfhydryl (SH) groups and lipoproteins in erythrocytes was measured by cytophotometry. In order to detect SH groups, blood smears were stained by M. Chevremont and J. Frederick's method [12], for lipoprotein detection the smears were stained by the method of M. Barenbaum [11]. Dry weight of peripheral blood erythrocytes was measured by interferometry [7]. Erythrocyte membranes were

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isolated using the method proposed by J. T. Dodge *et al.* [13]. Erythrocyte membrane protein composition was studied by disk electrophoresis in polyacrylamide gel by the method of Laemmli [14] in the presence of 0.1% sodium dodecylsulfate in a vertical system. The samples for scanning electron microscopy were prepared by the method of G. I. Kozinets and Yu. A. Simovart [5]. A total of 1000 cells from each examinee were counted using classification for quantitative characterization of red blood cells with different types of surface relief [5]. The preparations were examined and photographed under a JEM-100CXII (JEOL) electron microscope.

The following parameters of reversible erythrocyte aggregation were evaluated in blood microvolumes by vibration photometric method [9]: minimum mechanical resistance of erythrocyte aggregations (U_0, B), maximum mechanical resistance of erythrocyte aggregations (U_d, B), half-period of erythrocyte spontaneous aggregation (τc), and amplitude of photometrical signal characterizing the number of erythrocytes participating in reversible aggregation process (A, mm). The aggregation index $I_a=U_d/\tau$, characterizing the aggregation to disaggregation ratio, and integral coefficient of aggregation $K=(U_0U_dA)/\tau$ were calculated in arbitrary units.

The results were processed by methods of variation statistics. The significance of differences between the groups was evaluated using Student's *t* test and nonparametrical tests (Mann—Whitney's *U* test and Wander—Warden test).

RESULTS

Free-radical reactions play an important role in the development of pathological processes of different origin [2]. Lipid peroxides or products of their oxidative destruction can oxidize SH-containing compounds. Oxidative injuries accumulated during chronic diseases aggravate dysfunction of the antiradical defense system. Cytophotometry revealed lowered content of sulfhydryl groups and lipoproteins in circulating erythrocytes of patients with chronic bronchitis and chronic colitis compared to healthy individuals (Table 1). Decreased content of sulfhydryl groups and lipoproteins in peripheral blood erythrocytes of patients with chronic inflammatory diseases can promote aging and death of these cells.

Previous thin-layer chromatography and fluorescent probing studies revealed pronounced changes in lipid composition of erythrocyte membranes in patients with chronic inflammatory diseases. These changes were paralleled by increased viscosity of the lipid bilayer and considerable modification of the surface layers of erythrocyte membranes [10]. The basic characteristics of biological membranes, including erythrocyte membranes, are determined by both lipids and proteins [8]. Electrophoresis of erythrocyte membranes of patients with chronic inflammatory diseases showed decreased content of high molecular weight proteins (fractions 1, 2, 3) and increased content of proteins with molecular weight <60 kDa (fractions 4.5, 5, 6, 7, 8) in comparison with the parameters in normal controls (Table 2).

Oxidative capacity of protein components of erythrocyte membranes in patients with chronic inflammatory diseases can increase due to inhibition of antioxidative enzymes and reduced content of SH groups in red blood cells. Oxidative denaturation of erythrocyte membrane and cytoskeleton proteins (primarily spectrin, ankyrin, and band 3 protein) can be induced by increased content of LPO products, recorded in red blood cell membranes in chronic inflammatory processes. Oxidative protein denaturation, in turn, promotes their more intensive proteolytic degradation by endogenous proteases [8].

The state of erythrocyte protein cytoskeleton largely determines morphology of red blood cells [1]. Electron microscopy revealed accumulation of transitory, prehemolytical, and degenerating peripheral blood erythrocytes in the blood of patients with chronic diseases in comparison with normal subjects (Table 1).

Interferometry showed decreased mean dry weight of peripheral blood erythrocytes in patients (male and female) with chronic bronchitis, chronic inflammatory diseases of the upper respiratory tract, and chronic colitis compared to donors (Table 1). In the population of circulating erythrocytes the number of cells saturated with hemoglobin decreased, while the number of erythrocytes with low content of compact substances increased. Decreased content of compact substances in peripheral blood erythrocytes of patients with chronic inflammatory diseases indicates decreased activity of hemoglobin synthesis in the erythroid elements of the bone marrow.

Impaired membrane structure, disorders in erythrocyte metabolism and morphological abnormalities during the development of chronic inflammatory process determine enhanced aggregation capacity of cells and changes in blood rheology. Our experiments showed that erythrocyte aggregation activity in patients with chronic inflammatory diseases increased compared to healthy subjects (Table 3).

Hence, our findings indicate that disorders in the structure and metabolic status of erythrocytes are paralleled by an increase in the number of transformed cells and lead to changes in the functional characteristics of erythrocytes, specifically, increase their reversible aggregation. Changes in erythrocyte morpho-

TABLE 1. Concentrations of Sulfhydryl Groups and Lipoproteins (arb. units) and Dry Weight (ng) and Morphological Characteristics of Peripheral Blood Erythrocytes in Patients with Chronic Inflammatory Diseases ($\bar{X}\pm m$)

Parameter		Healthy	Patients with		
			chronic bronchitis	chronic inflammatory diseases of upper respiratory tract	chronic colitis
Sulfhydryl groups		0.351±0.007	0.319±0.009**	0.300±0.032****	0.271±0.026*
Lipoproteins		0.885±0.036	0.758±0.034***	0.849±0.111	0.703±0.083****
Dry weight	men	36.67±0.21	35.31±0.40**	32.41±0.81*	31.34±1.15*
	women	34.87±0.39	31.41±0.36*	31.56±0.80*	29.66±0.68*
Morphological forms of erythrocytes, %	discocytes	87.04±0.22	81.86±0.20*	83.18±0.45*	83.19±1.16**
	transitional	10.65±0.18	14.08±0.18*	12.83±0.38*	12.78±0.93****
	prehemolytical	2.18±0.05	3.69±0.09*	3.53±0.12*	3.56±0.26*
	degenerative	0.13±0.01	0.37±0.03*	0.46±0.04*	0.47±0.05*

Note. Morphological characteristics of erythrocytes as shown by scanning electron microscopy. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.02, ****p<0.05 compared to the control.

logy and function are paralleled by their enhanced hemolysis, which can be partially compensated by more intensive erythropoiesis, but the strain in the erythron (accumulation of cells with more rich surface relief is a morphological equivalent of this process) leads to the release of functionally defective cells into the bloodflow.

Universal type of disorders in the structural metabolic status and functional characteristics of erythrocytes in patients with chronic inflammatory diseases of different location are presumably determined by common mechanisms underlying these changes, which allows us to regard them as nonspecific signs of the involvement of the peripheral component of the

TABLE 2. Characteristics of Erythrocyte Membrane Protein Composition in Patients with Chronic Inflammatory Diseases ($\bar{X}\pm m$)

Parameter		Healthy	Patients with		
			chronic bronchitis	chronic inflammatory diseases of upper respiratory tract	chronic colitis
Total protein, mg/ml		17.06±1.19	18.00±1.64	18.25±1.51	16.67±0.93
Aggregated material, %		1.88±0.15	1.67±0.30	1.21±0.54	1.75±0.38
Protein fractions, %	1	9.26±0.19	9.06±0.32	7.38±0.44*	7.53±0.56
	2	10.96±0.28	8.25±0.61*	9.02±0.41*	8.95±0.71**
	2.1	3.51±0.17	3.94±0.30	3.01±0.26	3.41±0.19
	2.2	1.41±0.11	1.69±0.16	1.65±0.15	1.27±0.09
	3	21.91±0.42	20.92±0.80	19.24±1.14****	19.11±0.61**
	4.1	6.46±0.27	6.41±0.16	6.15±0.42	6.17±0.64
	4.2	6.50±0.26	7.22±0.10****	6.52±0.20	6.80±0.23
	4.5	11.77±0.31	12.60±0.37	13.42±0.49**	12.77±0.29
	4.9	4.24±0.25	3.79±0.25	3.83±0.20	4.30±0.13
	5	7.45±0.22	8.61±0.29**	7.95±0.22	7.97±0.37
	6	7.49±0.29	8.62±0.31***	7.67±0.45	8.94±0.47***
	7	4.42±0.37	5.11±0.37	7.53±1.00**	5.81±0.23***
	8	2.77±0.44	2.10±0.29	5.43±1.22****	5.23±0.99***

TABLE 3. Parameters of Reversible Aggregation of Peripheral Blood Erythrocytes in Patients with Chronic Inflammatory Diseases ($\bar{X}\pm m$)

Parameter	Healthy	Patients with		
		chronic bronchitis	chronic inflammatory diseases of upper respiratory tract	chronic colitis
U_o, B	13.43±0.51	18.10±1.90**	11.10±1.19	11.21±1.00
U_d, B	79.00±3.74	77.70±4.39	71.80±2.96	82.64±2.29
A, mm	29.17±0.76	40.10±2.93*	36.60±1.00**	36.93±3.51**
τ, sec	37.93±2.75	15.00±4.06**	17.20±2.24***	15.71±1.97**
K, arb. units	1184.44±151.22	4865.35±1172.87***	1809.99±280.30	2452.66±526.03***
I_a, arb. units	2.75±0.30	6.43±1.23***	4.40±0.48***	5.85±0.82**

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the control.

erythron in the intricate complex of whole body disorders, associated with the development of chronic inflammation.

Deficit of energy production and activation of LPO processes underlie abnormalities of membrane structure. Intensification of cell membrane LPO leads to shrinkage or destruction of the lipid bilayer, increases its microviscosity, decreases the area of protein-lipid contacts, impairs functional activity of enzymes, changes membrane permeability and surface charge, and disorders function of the membrane-receptor complex. LPO acts as a triggering mechanism increasing availability of lipid and protein membrane components for phospholipases and proteases, respectively. Decreased content of macroergic compounds is associated with intracellular accumulation of Ca^{2+}, because depletion of ATP pool leads to inactivation of ionic pumps and leakage of Ca^{2+} from extracellular space, as well as to activation of membrane-bound phospholipases, hydrolysis of phospholipids, and increase of membrane permeability.

Inadequate energy supply to the cells results in inhibition of their antioxidant systems and activation of LPO processes, which leads to oxidative destruction of cells, including erythrocytes. Decreased content of thiols and lipid antioxidants is usually associated with activation of LPO and, vice versa, more intense production of free radicals and LPO leads to a decrease in the content of lipid antioxidants and thiols. Increased LPO in cell is associated with impairment of the phospholipid-protein bonds, paralleled by an increase in phospholipase activity. Free fatty acids formed during phospholipolysis serve as substrates for LPO. Along with LPO activation, accumulation of Ca^{2+} (secondary messenger transferring the signal into cells) in erythrocytes triggers chain processes, *e.g.* activation of Ca^{2+}-dependent phospholipases and proteases leading to impairment of membrane structure, metabolism, ionic homeostasis, and to impairment of the cell shape and function.

The role of red blood cells in the body is not confined to their gas-transporting function. They participate in thrombus formation, regulation of acid-base balance and water-salt metabolism, in immune reactions, in deposition, transport, and metabolism of hormones and neurotransmitters, in binding and transport of viruses, toxins, and drugs. New reports demonstrate mutual effects of erythron cells and immune system. Hence, the developing changes in the structure and function of red blood cells of different maturity can modify their role in processes involved in the maintenance of homeostasis at the organism level. Presumably, disorders in erythrocyte membrane structure develop in many cells of the body, including cells involved in the regulation of erythropoiesis, during development of a chronic inflammatory process: in immunocompetent cells, hemopoiesis-inducing microenvironment cells, oxygen-sensitive and erythropoietin-producing renal cells, *etc.*, which can impair their functional activity and aggravate injuries.

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