Antioxidant Protection and Lipid Peroxidation in the Lymph and Blood during Complete Freund's Adjuvant-Induced Low-Grade Fever and Pyrogenal-Induced Fever

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Experiments on rats showed that complete Freund's adjuvant-induced low-grade fever and pyrogenal-induced fever of different duration are accompanied by an increase in the content of lipid peroxidation products and components of the antioxidant system not only in blood plasma, but also in the central lymph. These changes reflect activation of drainage, evacuation, and transport function of the lymphatic system. We conclude that the lymphatic system plays an important role in the maintenance of the prooxidant—antioxidant balance under these pathological conditions.

Key Words: *low-grade fever; fever; lipid peroxidation; antioxidant system; lymph; blood*

Free radical processes, including lipid peroxidation (LPO), are involved in the realization of various reactions. These processes may have positive and negative effects. The positive effects include the role of free radicals in the regulation of cell membrane permeability, nerve impulse conduction, destruction of damaged mitochondria, oxidative phosphorylation in mitochondria, mitogenesis, activity of lipid-dependent membrane-bound enzymes, synthesis of prostaglandins, leukotrienes, and deoxyribonucleotides, and microbicidal properties of the organism. Free radicals play a key role in the pathogenesis of radiation injury, tissue destruction, postischemic, reperfusion, and hyperoxic damages, some cardiovascular and genetically determined diseases, and intoxication [2,7]. It should be emphasized that LPO mainly occurs in the lipid bilayer of membranes. Damage to the lipid bilayer is one of the main causes of dysfunction in the cell and whole organism ("membrane disease") [11,12]. Constant formation of prooxidants in the organism is compensated by activity of intracellular and extracellular antioxidants, which contributes to the optimal prooxidant/antioxidant balance. However, little is known about the content of LPO products and components of the antioxidant system (AOS) in the lymph under pathological conditions [6].

Here we compared the content of LPO products and AOS components in the lymph of the thoracic duct (TD) and plasma from rats with low-grade fever and pyrogenal-induced fever of different duration.

MATERIALS AND METHODS

Experiments were performed on 103 albino rats. Low-grade fever was induced by injection of complete Freund's adjuvant (CFA, single dose 0.15 ml; Difco laboratories) with 0.1% killed and dried *Mycobacterium butyricum* into the pads of both hindlimbs. This treatment was conducted in accordance to the requirements for prolonged temperature response. Fever was

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induced by intramuscular injection of pyrogenal in a daily dose of 100 μg/kg for 3 and 10 days. Control rats (control-1, control-2, and control-3) received an equivalent volume of apyrogenic solution under similar conditions. The animals were anesthetized with sodium ethaminal in a dose of 50 mg/kg. The lymph was obtained by puncture of TD ostium (close to the opening into the venous angle). The blood was taken from the inferior vena cava. The animals were euthanized by a narcotic drug in the lethal dose. The amount of malonic dialdehyde (MDA) [3], lipid hydroperoxides [5], and nitric oxide (NO) [4], antioxidant potential (ceruloplasmin concentration) [15], activities of superoxide dismutase (SOD) [15], catalase [10], and peroxidase [13], and total antioxidant activity [1] were measured in biological fluids. The results were analyzed by Student's *t* test.

RESULTS

CFA-induced low-grade fever and pyrogenal-induced fever were accompanied by activation of LPO and AOS (Tables 1 and 2). On the one hand, the intensity of these processes in the central lymph was higher than in the blood. On the other hand, the degree of changes in biological fluids was higher in animals with low-grade fever (as compared to fever). For example, the amount of MDA (molecular product of LPO) in the lymph and blood plasma increased by 2 times in rats with low-grade fever. Threefold injection of pyrogenal was followed by a 66% increase in MDA content. MDA content in the lymph and blood was elevated by 69 and 31%, respectively, after 10-fold treatment with pyrogenal. After CFA injection, the amount of lipid hydroperoxides in the lymph and blood increased by 85 and 60%, respectively. The increase in the amount of lipid hydroperoxides in biological fluids was similar and did not depend on the duration of fever. The concentration of NO (marker of peroxidation) in the lymph and blood increased most significantly after 10-fold treatment with pyrogenal (by 5.4 and 3.8 times, respectively). Studying the components of AOS showed that CFA-induced low-grade fever is accompanied by a significant increase in total antioxidant activity (by 87%) and activities of SOD (by 85%) and catalase (by 81%) in the lymph. The most significant changes in peroxidase activity were observed in blood plasma from these rats (increase by 89%). Threefold treatment with LPS was followed by an increase in catalase activity (by 2.2 times), ceruloplasmin concentration (by 94%), and total antioxidant activity (by 83%) in

TABLE 1. Content of LPO Products and AOS Components in TD Lymph from Rats with CFA-Induced Low-Grade Fever and Pyrogenal-Induced Fever (*M*±*m*)

Parameter	Control-1	CFA administration	Control-2	Threefold treatment with pyrogenal	Control-3	Tenfold treatment with pyrogenal
MDA, μmol/liter	1.05±0.10	2.18±0.20*	1.03±0.05	1.69±0.05*	1.04±0.07	1.76±0.07*
	n=6	*n*=6	*n*=6	*n*=6	*n*=6	*n*=7
Lipid hydroperoxides, rel. units/ml	3.66±0.36	6.79±0.45*	3.19±0.44	4.96±0.18**	3.83±0.36	5.66±0.26**
	n=6	*n*=6	*n*=6	*n*=7	*n*=6	*n*=7
NO, μmol/liter	18.20±1.17	32.46±0.79*	17.16±1.81	22.83±2.36*	17.91±1.86	97.04±3.15*
	n=7	*n*=9	*n*=7	*n*=9	*n*=7	*n*=7
Total antioxidant activity, %	21.49±3.47	40.16±1.76*	21.17±2.31	38.77±1.56*	21.97±3.52	37.16±1.36*
	n=6	*n*=7	*n*=6	*n*=6	*n*=5	*n*=6
SOD, arb. units	0.95±0.03	1.76±0.02*	1.12±0.02	1.39±0.06**	1.06±0.04	0.61±0.03*
	n=7	*n*=10	*n*=6	*n*=8	*n*=6	*n*=7
Catalase, $\times 10^5$ μcat/liter	0.32±0.02	0.58±0.06*	0.29±0.03	0.65±0.04*	0.34±0.03	0.47±0.05*
	n=7	*n*=7	*n*=6	*n*=8	*n*=6	*n*=7
Peroxidase, U	116.29±3.71	146.76±3.59*	113.62±2.89	155.81±6.45*	117.63±0.52	199.10±11.37*
	n=6	*n*=6	*n*=6	*n*=6	*n*=7	*n*=7
Ceruloplasmin, μmol/liter	1.48±0.17	2.25±0.11**	1.36±0.12	2.64±0.19*	1.43±0.15	3.62±0.16*
	n=6	*n*=7	*n*=6	*n*=7	*n*=7	*n*=7

Note. Here and in Table 2: *n*, number of animals. *$p<0.001$, **$p<0.01$, and ***$p<0.05$.

the TD lymph. We revealed only the increase in plasma peroxidase activity at this stage of the study (by 2.4 times). The increase in the concentration of other components of AOS was less pronounced (<45%). Ten-day pyrogenal-induced fever was accompanied by an increase in the concentration of ceruloplasmin in the lymph and blood (by 2.5 times and 56%, respectively). Antioxidant activity and peroxidase activity in the lymph were elevated by 69%. The total antioxidant activity remained unchanged in blood plasma. Peroxidase activity in the plasma increased by 2.2 times. It should be emphasized that SOD activity in the lymph and blood was reduced by 42.5 and 31.5%, respectively, after 10-fold treatment with pyrogenal. Moreover, the concentration of ceruloplasmin did not change in animals with low-grade fever and 3-day pyrogenal-induced fever.

Activation of LPO is one of the major causes of cell damage. Reactive oxygen metabolites rapidly interact with membrane phospholipids, which results in a chain reaction of radical formation, increase in the degree of cell destruction, and activation of the major enzyme reactions. Reactive oxygen metabolites are formed during the synthesis of leukotrienes and prostaglandins, autooxidation of catecholamines, and various reactions induced by transition metals [2,7]. The increase in body temperature of different duration and severity provides conditions for LPO activation. They include the production of prostaglandins (mediators of fever) and leukotrienes, hypercatecholaminemia, *etc*. It was accompanied by an increase in the content of LPO products (MDA and lipid hydroperoxides) in the lymph and blood. The increase in glucocorticosteroid concentration and catabolic (in lymphoid tissue) and prooxidant effects of these substances also contribute to the prevalence of peroxidation processes in the lymphatic system of specimens with low-grade fever and pyrogenal-induced fever. However, hyperactivation of peroxidation determine the development of microcirculatory disturbances, metabolic changes, and progression of hypoxia. It should be emphasized that hypoxia promotes induction of LPO. The observed changes contribute to a "vicious cycle" with impairment of homeostasis and bioenergetics. Long-term persistence of these changes causes severe dysfunction. The considerable increase in the contents of MDA and lipid hydroperoxides in the TD lymph from animals with a CFA-induced low-grade fever and fever reflects the activation of drainage-and-evacuation and transport functions of the lymphatic system (relative to LPO products) under pathological conditions. Activation of AOS in the lymph reduces circulation of toxic LPO products. Our experiments showed that SOD activity in the lymph and blood plasma decreases

TABLE 2. Content of LPO Products and AOS Components in Blood Plasma from Rats with CFA-Induced Low-Grade Fever and Pyrogenal-Induced Fever (*M*±*m*)

Parameter	Control-1	CFA administration	Control-2	Threefold treatment with pyrogenal	Control-3	Tenfold treatment with pyrogenal
MDA, μmol/liter	1.58±0.10 *n*=6	3.00±0.41** *n*=7	1.50±0.10 *n*=6	2.49±0.11* *n*=7	1.61±0.13 *n*=6	2.11±0.09** *n*=7
Lipid hydroperoxides, rel. units/ml	8.02±0.33 *n*=6	12.85±0.74* *n*=7	7.50±0.43 *n*=6	11.34±0.39* *n*=6	8.04±0.30 *n*=6	12.39±1.23** *n*=7
NO, μmol/liter	27.43±1.24 *n*=7	38.61±1.29* *n*=10	27.13±1.12 *n*=7	40.90±1.13* *n*=10	29.31±1.83 *n*=7	113.42±2.44* *n*=8
Total antioxidant activity, %	45.04±1.95 *n*=7	59.01±5.09*** *n*=7	46.34±1.92 *n*=5	54.26±2.70*** *n*=7	45.61±1.67 *n*=5	49.83±1.51 *n*=7
SOD, arb. units	1.00±0.04 *n*=7	1.51±0.05* *n*=9	1.06±0.01 *n*=7	1.41±0.05* *n*=9	1.08±0.06 *n*=8	0.74±0.04* *n*=7
Catalase, $\times 10^5$ μcat/liter	6.80±0.22 *n*=9	10.38±0.14* *n*=9	6.9„ 0.16 *n*=9	9.95±0.13* *n*=9	6.75±0.37 *n*=7	12.22±0.19* *n*=10
Peroxidase, U	138.11±4.92 *n*=6	261.29±11.41* *n*=8	126.28±5.84 *n*=6	310.59±13.42* *n*=8	129.18±8.19 *n*=6	281.86±18.20* *n*=8
Ceruloplasmin, μmol/liter	4.25±0.15 *n*=7	4.79±0.07 *n*=7	4.14±0.16 *n*=7	3.97±0.15 *n*=9	4.43±0.19 *n*=7	6.92±0.36* *n*=8

after 10-fold treatment with pyrogenal. SOD is one of the key enzymes of antioxidant protection, which catalyzes the reaction of oxygen dismutation in H_2O_2. Moreover, activities of catalase and peroxidase in the lymph increased less significantly than in the blood. These changes illustrate depletion of AOS. However, the concentration of NO increased most significantly after 10-fold treatment with pyrogenal. Previous studies showed that the molecule of NO binds to the cytosolic form of guanylate cyclase, which is followed by production of cyclic guanosine monophosphate. This secondary messenger triggers the protein kinase G-mediated activation of SOD [8]. NO plays a key role in the formation of highly reactive oxygen species (*e.g.*, peroxide radical) that possess the neurotoxic properties. It may cause damage and death of neurons. Previous studies demonstrated activation of LPO and increased production of NO in rat pups with febrile convulsions [9]. We showed that 10-day fever is accompanied by a significant increase in ceruloplasmin concentration in the TD lymph. CP belongs to a group of chelate compounds, which bind transition metal ions and prevent their involvement in peroxide decomposition. The observed changes serve as a compensatory response, which is directed towards the maintenance of AOS.

We conclude that the lymphatic system plays an important role in the maintenance of the prooxidant-antioxidant balance in specimens with a CFA-induced low-grade fever and pyrogenal-induced fever.

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