Effect of Dietary Supplementation with Selenium and Vitamin E on Certain Immunological Parameters in Rats

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It is shown that enriching the rat diet with selenium and vitamin E augments the generation of spleen antibody-producing cells and the T-cell blast-transformation response to mitogen. The addition of selenium alone fails to affect the immune reaction; however, the addition of vitamin E alone boosts the T-cell response to mitogen. Dietary supplementation with selenium and/or vitamin E has no marked effect on the function of peritoneal macrophages or on lipid peroxidation.

Key Words: *selenium; vitamin E; immunity; peritoneal macrophages*

The discovery of the mechanisms of biological activity of selenium (Se) as an essential trace element prompted extensive investigations of its effect on the immune status of the organism [14]. The immunomodulatory effect of Se compounds is attributed to the functioning of Se-containing glutathione peroxidases, which act to reduce hydroperoxides and other products of free-radical chain reactions and regulate the output of arachidonic acid lipoxygenase and cyclooxygenase metabolites [15]. In view of the diversity of Se-containing proteins in mammalian tissues [6], investigators do not rule out other possible mechanisms of Se effects on the processes of vital activity [7].

Vitamin E, an integral component of biological membranes, regulates the level of membrane-associated lipid peroxidation, which has a marked influence on the functional state of cells [9,11]. Despite the existence of phenomenological data pointing to an immunomodulatory effect of Se and vitamin E, the question of a combined effect of a supplementary (as compared to the physiological level) amount of these antioxidants has been little studied. Therefore, the goal of the present investigation was to study the effects of Se- and vitamin E-supplemented diets on the parameters of humoral and cellular immunity, as well as on the functional-metabolic activity of peritoneal macrophages in rats.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 143±6.4 g. The animals were distributed into four groups and during one month received an isocaloric diet consisting of casein (20% of caloric content), cornstarch (56% of caloric content), vegetable oil and lard in a ratio of 1:9 (24% of caloric content), and wheat bran (0.8 g per rat every day). All diets included the necessary vitamins and minerals. The Se level in the products used was about 0.192 mg/kg food. The diet contained 0.14 mg α-tocopherol and 0.1 g polyunsaturated fatty acids per rat per day, so that the animals received the full physiological requirement of vitamin E. The diets of experimental groups (Se-1 and Se-2) were supplemented with Se-containing yeast (Alko) at a rate of 2 mg Se/kg diet. The animals of the control groups (C-1 and C-2) did not receive additional Se. Rats of the Se-2 and C-1 group were given an increased dose

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TABLE 1. Body Weight and Relative Organ Weight of Rats on Different Experimental Diets ($M \pm m$)

Group	Dietary content		Body weight, g	Organ indexes, %		
	Vitamin E, mg per rat per day	Se, mg/kg diet		liver	spleen	thymus
C−1	0.64	0.192	237±4.81	3.13±0.09	0.27±0.03	0.11±0.02
C−2	0.14	0.192	260±10.58	2.84±0.07	0.27±0.02	0.13±0.02
Se−1	0.14	2.192	266±10.20	3.09±0.11	0.33±0.03*	0.13±0.01
Se−2	0.64	2.192	251±13.47	3.19±0.09	0.26±0.02	0.14±0.01

Note. *: Se−1 group reliably differs from C−1 and C−2 groups ($p<0.05$).

of vitamin E (0.64 mg per day), which is ten times the physiological standard (0.6 mg vitamin E per gram of polyunsaturated fatty acids). Both food and water were given *ad libitum*.

Animals were sacrificed by decapitation under ether anesthesia. The liver, spleen, and thymus were extracted in order to estimate their relative weight. The level of lipid peroxidation was evaluated by recording the content of diene conjugates in the serum [4], the content of thiobarbituric acid-reacting products in the serum [1], and the content of group lymphoid follicles of the small intestine and liver [12].

For the assessment of functional status of the immune system, animals were immunized intraperitoneally with a 20% suspension of sheep red blood cells. The immune response was recorded 5 days later. The status of B-cell immunoreactivity was judged by the dynamics of serum hemagglutinin titers [3] and the number of antibody-producing cells per spleen, this being determined by the method of local hemolysis in liquid medium, i.e., the count of plaque-forming cells [8]. The number of T lymphocytes in the peripheral blood was estimated by the method of spontaneous rosette formation with sheep red blood cells (rosette-forming cells - RFC) [5]. The functional activity of T cells was studied in the blast-transformation reaction (BTR) of blood lymphocytes in response to phytohemagglutinin [2]. The functional-metabolic activity of peritoneal macrophages was assessed in a monolayer culture by reduction of nitro blue tetrazolium (NBT test) [13].

Results were evaluated using the method of variational statistics with Student's test.

RESULTS

The rats consuming the experimental and control diets did not differ significantly in such parameters as body weight and relative weight of liver and thymus. In the group that received the Se-enriched diet without additional supplementation with vitamin E (Se-1 group) a reliable increase of spleen relative weight was observed (Table 1).

The values of parameters characterizing the level of lipid peroxidation are presented in Table 2. It may be concluded that under the given conditions, Se and/or vitamin E supplementation failed to induce detectable changes in the lipid peroxidation level.

Hemagglutinin antibody titers did not differ reliably in animals of the control and experimental groups. However, rats on the Se- and vitamin E-enriched diet exhibited a reliably elevated level of spleen plaque-forming cells (Table 3).

The status of cellular immunity was assessed by the number of T lymphocytes (RFC) in the peripheral blood and their mitogenic activity (BTR). Despite the similar quantity of T lymphocytes in animals of different groups, the functional activity was highest in the rats consuming high

TABLE 2. Diene Conjugate Content in the Serum and Thiobarbituric Acid−Reacting Product Content in Rat Serum, Liver, and Group Lymphoid Follicles of Small Intestine ($M \pm m$)

Group	Serum		Group lymphoid follicles	Liver
	diene conjugates, arb. units	malonic dialdehyde, nmol/g	malonic dialdehyde, nmol/g	malonic dialdehyde, nmol/g
C−1	1.79±0.10	10.3±0.9	93.2±11.0	399.2±25.5
C−2	1.74±0.12	10.2±1.1	87.2±6.9	366.7±19.8
Se−1	1.61±0.09	9.7±0.7	101.2±9.8	380.2±27.2
Se−2	1.74±0.14	10.2±0.7	102.6±7.7	379.9±29.5

TABLE 3. Indexes of Immunoreactivity of Rats Fed with Experimental Diets ($M \pm m$)

Group	Antibody titer, log	RFC, %	PFC number per 10^6 splenocytes	BTR, %	NBT test, arb. units
C−1	3.47±0.13	18.5±1.29	65.0±4.43	38.2±2.49**	0.27±0.02
C−2	4.05±0.22	26.7±2.90	65.0±4.23	16.6±4.42	0.26±0.02
Se−1	3.93±0.11	24.83±3.55	65.8±2.42	16.5±3.87	0.26±0.01
Se−2	4.05±0.11	29.83±3.39*	66.7±0.16	38.5±1.77**	0.29±0.02

Note. *: C−1 and Se−2 groups differ reliably ($p<0.05$); **: the same concerning comparison of C−1 and Se−1 groups, as well as C−2 and Se−2 groups (in both cases $p<0.05$).

amounts of vitamin E (C-1 and Se-2 groups). Reliable differences in this parameter were found when groups Se-1 and C-1, as well as Se-2 and C-2 were compared (Table 3). This is apparently due to conformational restructuring of the lymphocyte membranes following increased intake of vitamin E in the organism [10].

Since cells of the mononuclear phagocyte system play an important role in immune functioning, producing lipid hydroperoxide compounds, superoxide anion, hydrogen peroxide, hydroxyl radicals, etc., we studied the effect of the antioxidants in question on the functional-metabolic activity of peritoneal macrophages. Assessment by NBT test failed to reveal reliable differences between animals of the experimental and control groups (Table 3). This is in agreement with data presented in Table 2 indicating an absence of differences in the generation of lipid peroxidation products.

Thus, combined supplementation of the rat diet with Se-containing yeast and vitamin E stimulates the B-cell system of immunity, as expressed in a rise of the number of spleen antibody-producing cells, and enhances the reaction of T cells to mitogen. Separate supplementation with either nutrient results in a less pronounced immunostimulation, manifested mainly in increased functional activity of T lymphocytes induced by the elevated content of vitamin E.

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