by Igk-1^b-pulsed APC of the responding line of August rats and the nonresponding WAG line. PPD-puled APC of these lines were used as the positive control.

The results of these experiments showed (Fig. 3) that only presentation of Igk-1^b by cells of August, but not of WAG, rats induced specific stimulation of immune (WAG × August)F_1 T lymphocytes. August and WAG APC were equally effective under these circumstances in presentation by the T cells of another AG, namely PPD, the response to which was comparable in rats of these two lines (Table 1).

The results thus demonstrate the MHC-dependent Ir gene-control of the stage ofmacrophage–T cell interaction in the course of recognition of the Igk-1^b allotype by T lymphocytes. They also show that the Igk-1^b alloantigen is recognized by T cells in association with characteristic MHC products. Absence of immunoreactivity to Igk-1^b in rats may accordingly be due to deficiency of clones of RT-1^u-restricted (WAG) T lymphocytes specific for Igk-1^b, and to ability to form functional complexes of Igk-1^b and RT-1^u products at the APC level (AG-specific presentation defect) or to generation of RT-1^u-restricted Igk-1^b-specific T suppressors (WAG).

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EFFECT OF VITAMIN E ON OXIDATIVE METABOLISM OF MACROPHAGES

A. T. Sharmanov, B. B. Aidarkhanov, and S. M. Kurmangaliev

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Adaptation of polymorphonuclear leukocytes (PNL), macrophages, and lymphocytes is accompanied by a respiratory "burst," characterized by intensification of oxidative metabolism and release of active oxygen radicals [3, 10]. The latter are largely responsible for the bactericidal properties of phagocytes but they may be toxic for surrounding tissues and for the cells themselves [7, 9, 13]. The important role of the enzyme superoxide dismutase (SOD), a modulator of oxidative metabolism of PNL which possesses anti-inflammatory properties, was demonstrated previously [9]. Free-radical reactions are inhibited by antioxidants and, in particular, by vitamin E, which blocks H_2O_2 production by PNL [4]. However, the mechanism of this phenomenon is largely unexplained.

The aim of this investigation was to study oxidative metabolism of macrophages under conditions of vitamin E deficiency, characterized by activation of lipid peroxidation (LPO) *in vivo*.

METHODS

Experiments were carried out on growing male Aug-Lac rats weighing initially 40–60 g and kept for 2 months on a semisynthetic balanced diet of the following composition (in %): protein 24.5 (casein 21, wheat gluten 3.5), carbohydrate 59.4 (starch 53.5, sucrose 5.9), fat 10

Kazakh Branch, Institute of Nutrition, Academy of Medical Sciences of the USSR, Alma-Ata.

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TABLE 1. Luminol-Dependent ChL of Macrophages Stimulated by Opsonized Zymosan under Conditions of Vitamin E Deficiency (M ± m)

Experimental conditions	Maximal reaction velocity, mV/min	Integral parameter, mV	
		for 10 min	for 20 min
Control	4,6±1,2	270±70	715±150
Vitamin E deficiency	14,9±3,8*	790±185*	1710±340†

Legend. *P < 0.05, †P < 0.02 compared with control.

TABLE 2. Activity of Antioxidant Enzymes in Vitamin E Deficiency (M ± m)

Experimental conditions	SOD, units/10^6 cells	GP, nmoles NADPH/10^6 cells	GR, nmoles NADPH/10^6 cells
Control	6,4±1,3	91,6±16,1	17,5±3,6
Vitamin E deficiency	3,3±0,8	83,7±11,0	16,6±1,1

(lard 8, linetol 2), cellulose 1.5, mixed salts and essential quantities of vitamins [12]. The animals were divided into two groups: 1) vitamin E added to the diet in a dose of 100 mg/kg diet (control), 2) rat not receiving vitamin E. To obtain macrophage-enriched peritoneal exudate, the animals were given an intraperitoneal injection of 100 ml of 4% peptone 3 days before the experiment. To obtain macrophages the peritoneal cavity was washed out with 40 ml of medium 199 with hepatin (20 U/ml). Any contaminating erythrocytes were hemolyzed with 0.87% NH_4Cl for 20 min at 4°C. Heparinized blood, taken by cardiac puncture, was centrifuged at 400 g. The plasma was mixed with zymosan and opsonized for 1 h at 37°C. The respiratory "burst" of the macrophages was assessed by luminol-dependent chemiluminescence (ChL) on the LKB-1250 WALLAC luminometer. A suspension of macrophages containing $2 \cdot 10^6$ cells in 400 µl of medium 199 was introduced into plastic cuvettes to which were added about 400 µl of luminol ($2.8 \cdot 10^{-5}$ mole/liter) and 10^8 zymosan particles. ChL was recorded every 10 sec for 20 min.

The results were expressed in millivolts per $2 \cdot 10^6$ macrophages.

To determine activity of "antioxidant" enzymes in the macrophages, the cells were solubilized in 0.1% Triton X-100 and homogenized in a Teflon Potter-Elvehjem homogenizer. SOD activity was estimated in the xanthine oxidase reaction by measuring the degree of reduction of nitro-BT to blue diformazan [6]. The measurements were made on a Hitachi-557 spectrophotometer at 540 nm. Glutathione reductase (GF) activity was determined at 340 nm on the basis of the quantity of NADPH used in the reduction of oxidized glutathione [14]. Glutathione peroxidase (GP) activity was determined in a coupled reaction with GR at 340 nm, with cumene hydroperoxide used as the substrate [11].

The microviscosity of the lipid phase of the macrophagal plasma membrane was estimated as the degree of excimerization of pyrene, which was added in quantities of 1 and 2 µl of a 5 mM alcoholic solution to a cell suspension containing $2 \cdot 10^6$ macrophages in 250 µl of medium 199. Fluorescence of pyrene was excited at 334 nm and recorded at 392 nm (monomers) and 475 nm (excimers) with excitation and emission slits 2 and 5 nm wide respectively. The length of the optical path in the quartz cuvette was 5 mm. Measurements were made with the Hitachi-850 fluorometer.

The following reagents were used: Luminol, zymosan A, pyrene, and curene hydroperoxide were from Sigma, USA; xanthine oxidase and SOD were from Calbiochem, USA; xanthine, catalase, D,L-α-tocopherol, and Triton X-100 were from Serva, West Germany; GR, oxidized and reduced glutathione, and NADPH were from Boehringer-Mannheim, West Germany; the other reagents were of Soviet manufacture and of the chemically pure and highly pure grades.

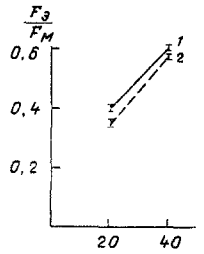

Fig. 1. Degree of excimerization of pyrene in macrophages in vitamin E deficiency. Abscissa, pyrene concentration (in μM). 1) Control; 2) vitamin E deficiency. F_e) Fluorescence of excimers; F_m) fluorescence of monomers.

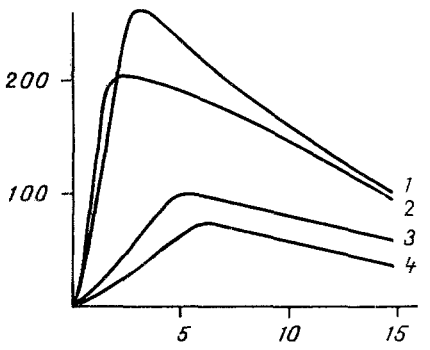

Fig. 2. Inhibition of luminol-dependent ChL of macrophages by α-tocopherol. Abscissa, time (in min); ordinate, intensity of ChL (in mV). 1) Control; 2, 3, 4) α-tocopherol in concentrations of $1.6 \cdot 10^{-7}$, $1.6 \cdot 10^{-6}$, and $1.6 \cdot 10^{-5}$M, respectively.

RESULTS

The respiratory "burst" of macrophages, stimulated by opsonized zymosan, was estimated on the basis of three parameters characterizing the resultant profile of luminol-dependent ChL: maximal reaction velocity — the ratio of the amplitude of the response to the time taken to reach the peak, reflecting the degree of activation of the macrophages — and also integral parameters for 10 and 20 min, estimated by the formula

$$(\Sigma mV\frac{1}{6}) \cdot 10 \text{ or } (\Sigma mV\frac{1}{6}) \cdot 20 \quad (5)$$

Vitamin E deficiency caused an almost threefold increase in ChL of the macrophages. The kinetics of the response was shifted toward increased activation of the cells (Table 1). Release of oxygen radicals by macrophages is known to be connected with activation of membrane-bound enzymes of the NADPH-oxidase system [3]. The degree of activation of the cells is largely dependent on the state of the receptor apparatus of the plasma membrane, responsible for binding with the ligands, or in this case with opsonized zymosan [2]. All these parameters depend on the physicochemical state of the cell membranes. In vitamin E deficiency we observed an increase in microviscosity of the lipid phase of the macrophagal membranes (Fig. 1). In deficiency of antioxidants and, in particular, of vitamin E, LPO was stimulated, causing changes in the fatty-acid composition and increased viscosity of the cell membranes [1].

It was shown previously that luminol-dependent ChL of macrophages, stimulated by opsonized zymosan, is due to oxidation of the luminol to the thalate ion under the influence of the superoxide radical and hydrogen peroxide [5]. In vitamin E deficiency increased generation mainly of the above-mentioned oxygen radicals, which are metabolized by SOD and catalyze, evidently takes place. Luminol-dependent ChL of macrophages is completely blocked by the addition of SOD in a dose of 29.8 units/ml, by the addition of SOD in a dose of 29.8 units/ml, and catalase (130,000 units) inhibits the reaction by 78%.

In vitamin E deficiency a tendency was observed for SOD activity in the macrophages to be reduced (Table 2). Activity of other antioxidant enzymes (GP and GR) was unchanged.

Incubation of macrophages with vitamin E for 6 h led to significant inhibition of luminol-dependent ChL (Fig. 2). It was shown previously that vitamin E causes increased ingestion of particles by PNL and a decrease in their bactericidal power. The cause of this dissociation between the parameters of phagocytosis under the influence of antioxidants is evidently restriction of release of oxygen radicals, which play a key role in killing of bacteria.

Vitamin E deficiency thus leads to increased generation of oxygen radicals by macrophages, with a consequent increase in viscosity of the plasma membranes and subsequent functional changes. These changes are due to a decrease in the vitamin E concentration in the body but are not connected with activity of antioxidant enzymes.

Antioxidants, especially vitamin E, evidently play an important role in the regulation of oxidative metabolism of macrophages, thus facilitating the phagocytic reaction and protecting surrounding tissues as well as the cells themselves from peroxidation.

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