an intermediate stage of differentiation of Ts inducers, detected in the presence of tolerance, or whether they are the functional equivalents of Lyt $1^+,2^+$ Ts effectors [12], suppressing proliferation of Lyt 1^+ cells.

The data given in this paper are thus evidence that in the course of the immune response, during induction of DTH by intravenous injection of syngeneic spleen cells, modified by a hapten, parallel with the formation of effectors of DTH [5], the formation of two types of antigen-binding Ts, differing from one another in their Lyt phenotype and functional properties, takes place in the spleen of the experimental animals.

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LIPID PEROXIDATION IN THE RAT LIVER DURING STIMULATION OF MACROPHAGES

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KEY WORDS: mononuclear phagocyte system; lipid peroxidation; zymosan; liver

Most tumoricidal and bactericidal effects of macrophages are known to be connected with the ability of these cells to produce active forms of oxygen (the superoxide radical, hydrogen peroxide, the hydroxyl radical, and singlet oxygen) [14]. Phagocytosis and various surface-active factors, including adjuvants, chemotaxic peptides, lectins, endotoxins, and so on, lead to characteristic metabolic changes in macrophages, accompanied by a "burst" of oxygen consumption and by an increase in the production of reactive metabolites of oxygen [6]. Considering the high ability of activated forms of oxygen to initiate free-radical lipid peroxidation (LPO) reactions in membranes, and also their free liberation into the extracellular medium [8], it is logical to suggest that induced macrophages participate in the realization of the pro-oxidant effect *in vivo*. Nevertheless, the protective action of zymosan, a stimulator of the mononuclear phagocyte system (MPS), under conditions of lesions of radiation or free-radical nature is difficult to explain from this point of view [1]. In the present investigation accumulation of one end product of LPO (malonic dialdehyde, MDA) in the animal

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liver was estimated at different stages of disinhibition of MPS by zymosan, a complex biopolymer of the yeast cell membrane.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male Wistar rats weighing 160-180 g. Under superficial ether anesthesia a 0.5% suspension of zymosan in sterile physiological saline was injected intravenously into the animals in a dose of 25 mg/kg. Anesthetized control animals were given an injection of physiological saline. Phagocytic clearance function was investigated in the rats 1, 3, 7, and 14 days after the injection. For this purpose the animals were given an intravenous injection of a suspension of colloidal carbon (from G. Wagner, West Germany) in a dose of 200 mg/kg. Blood samples were taken 15-20 sec and 1, 3, 6, and 10 min after loading and transferred into a 0.1% solution of sodium carbonate containing heparin (5 U/ml). The carbon concentration was determined by measuring the optical density of the mixture at a wavelength of 650 nm on a Specord M-40 spectrophotometer (East Germany). The half-elimination time ($T_{1/2}$) of the colloidal carbon from the blood stream was calculated.

The rats were decapitated 2 h later and pieces of liver were fixed in Carnoy's fluid. The total number of nonparenchymatous cells, including those loaded with carbon (per thousand hepatocytes), was determined in sections 4-5 μ thick, stained with hematoxylin and eosin, under a magnification of 1000.

The MDA concentration in the liver was determined spectrophotometrically [9]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of elimination of colloidal carbon from the blood stream, reflecting mainly the phagocytic clearance function of the Kupffer cells [2, 3], revealed that in the initial stages of stimulation of MPS the process gradually accelerated (Fig. 1). The greatest changes were observed on the 7th day after injection of zymosan into the animals. This was manifested as an almost threefold decrease in $T_{1/2}$. After two weeks the value of this parameter had returned to the control level.

The number of nonparenchymatous cells in the liver tissue was increased as early as on the first day after the injection (Fig. 2). During this period the number of cells loaded with carbon particles and the number of unloaded cells were about equal. Later (3rd-7th days) a further increase in the number of stromal cells was observed, mainly on account of unloaded cells. By the 7th day the changes in the structure of the cell population were most distinct: the ratio of loaded to unloaded cells was 1:2. This was because saturation of the liver macrophages with foreign particles led to secondary disinhibition of monocytopoiesis and to the arrival of their fresh precursors in the liver [11]. The size of the foci of mononuclear infiltration in the liver at this time was maximal, more than three times larger than those observed 24 h after injection of zymosan (14.85 ± 2.0 and 4.58 ± 0.53 relative units, respectively, $P < 0.001$). In the final stages of the experiment (14th day) the ratio of phagocytic cells to nonphagocytic had returned close to its initial level.

The accumulation of LPO products in liver tissue immediately after injection of zymosan showed phasic changes (Fig. 3). For instance, during the first day immediately after injection of zymosan particles a marked increase was observed in the MDA concentration in the liver. However, by the 3rd day, parallel with disinhibition of the liver macrophages, the value of this parameter fell to control levels. On the 7th day, corresponding to the peak level of activation of MPS (Figs. 1 and 2), however, the MDA concentration in the liver was lower than in intact animals. After 14 days, when the clearing function was back to normal and the number of nonphagocytic stromal cells was reduced, the MDA level returned to control values. Characteristically, by contrast with the values of the parameters in intact animals, in rats whose MPS was stimulated by zymosan, persistent negative correlation was formed between the MDA concentration in the liver tissue and the number of nonparenchymatous cells ($r_{xy} = -0.77$; $t_f = 6.1$), including the cells of the nonphagocytic pool ($r_{xy} = -0.82$; $t_f = 7.16$). Close positive correlation was observed between $T_{1/2}$ and the MDA level ($r_{xy} = 0.60$; $t_f = 6.97$).

To sum up the results, it can be stated that in response to injection of zymosan particles, initial activation of LPO may arise due both to more intensive formation of the superoxide radical from resident macrophages of the liver [7] and depression of the antiradical activity of the superoxide dismutase of the phagocytes under these circumstances [4]. Active

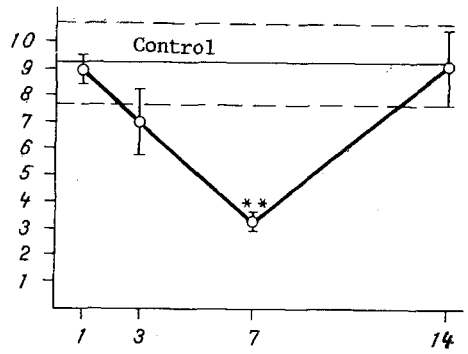

Fig. 1

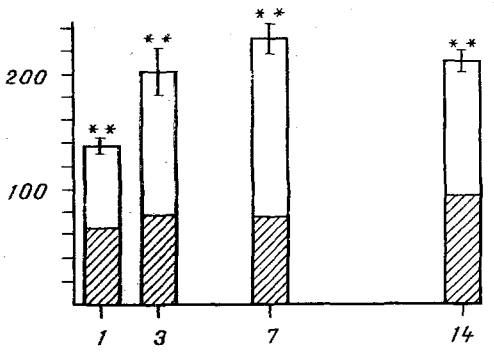

Fig. 2

Fig. 1. Half-elimination time of colloidal carbon from blood of rats at various times after injection of zymosan. Abscissa, time after injection of zymosan (in days); ordinate, $T_{1/2}$ (in min). Here and in Figs. 2 and 3: *P < 0.05, **P < 0.001 relative to control.

Fig. 2. Changes in number of nonparenchymatous cells and also in ratio between number of cells loaded with carbon particles (shaded part of column) and unloaded cells (unshaded part of column) in liver tissue after injection of zymosan. Abscissa, time after injection of zymosan (in days); ordinate, number of nonparenchymatous liver cells (in percent of control).

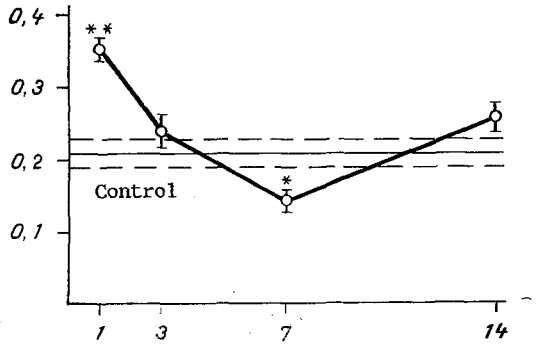

Fig. 3. MDA concentration in rat liver at different times after injection of zymosan. Abscissa, time after injection of zymosan (in days); ordinate, MDA level (in μmoles/mg tissue).

metabolites of oxygen, in doses not affecting structure or viability of the macrophages, subsequently disturbed the function of their receptor apparatus, thereby reducing the number of phagocytic cells [12]. Activity of the "antioxidative enzymes" in resting phagocytes, the proportion of which increased considerably in the course of this experiment, was increased [13]. The fall in the MDA level toward the time of maximal stimulation of MPS by zymosan and the increase in the pool of nonphagocytic cells may be the result of increased production of antioxidants, including reduced glutathione [10], by them. The presence of active transport systems for this tripeptide in the plasma membrane of the hepatocytes [5] enables it to participate in the glutathione system protecting the liver cells against oxidative damage.

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ACTION OF T-ACTIVIN ON ACTIVITY OF HUMAN NATURAL KILLER CELLS *IN VITRO*

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Investigations in recent years have shown that the thymus may exert its immunoregulatory function through a number of biologically active fractions [1], which can be used to correct disturbances of the T-system of immunity in the treatment of certain diseases.

This paper describes a study of the action of the Soviet preparation T-activin on activity of human natural killer cells (NKC) *in vitro*. Previous investigations showed [10] that NKC activity is depressed by the action of biologically active fractions from the thymus. However, the action of T-activin on NKC activity has not been studied. Evidence has been obtained that T-activin, under certain conditions, stimulates interferon production [2, 7], i.e., that it can stimulate NKC through the production of immune interferon.

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized blood of 19 healthy blood donors (without any immunologic disturbances) aged between 16 and 46 years, in a Ficoll–Verografin gradient [9]. The lymphocytes were washed twice with buffered physiological saline, after which suspensions of lymphocytes were prepared in complete nutrient medium based on medium RPMI-1640 (from Serva, West Germany), containing 10% embryonic calf serum with the addition of glutamine (200 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). The initial cell concentration was 10^7 lymphocytes/ml medium. The K-562 chronic human myeloid leukemia cells, cultured *in vitro*, used as targets were labeled with ^{3}H-uridine in a dose of 3 µCi/ml culture. In accordance with the modification of the ^{3}H-uridine method of determining NKC activity, developed in the laboratory of Cellular Immunopathology and Biotechnology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR [4], after preparation of the initial concentration of target cells, namely 10^5 cells/ml, and addition of pancreatic RNase to the system in a dose of 5 µg/ml, the lymphocytes and targets were distributed in a volume of 0.1 ml into round-bottomed cells of 96-well microplates (from Nunclon, Denmark). To investigate NKC activity, the serial dilutions principle developed in the Department of Immunology, N. I. Pirogov Second Moscow Medical Institute [5] was used (NKC activity was tested with effector and target cells in the ratio of between 100:1 and 6:1). T-activin (from the Laboratory of Molecular Immunology, N. I. Pirogov Second Moscow Medical Institute), in doses of 20, 2.5, 1.5, 1, and 0.5 µg/10^6 lymphocytes, was added to the lymphocyte suspension immediately before distribution into the wells. The cells were incubated for 14 h at 37°C in an atmosphere containing 5% CO_2, after which the contents of the wells were deposited on glass fiber filters with a pore diameter of 2.5 µ and harvested by means of a multichannel biological fraction collector (from Dyna-

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