T cells, effectors of antibody-dependent cytotoxicity, and mononuclear phagocytes. Since activated macrophages, together with natural killers, constitute the first line of nonspecific defense against malignantly transformed cells, lengthening of the latent period of appearance of tumors, which we noted in the present experiments, can be explained by the ability of CP to activate precisely this cell population.

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INHIBITION OF REACYLATION OF PHOSPHOLIPIDS DURING OXIDATIVE DAMAGE TO TUMOR CELLS

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KEY WORDS: oxidative stress; phospholipids; reacylation; arachidonic acid.

Tumor cells are exposed to oxidative stress in the tumor-bearing host during interaction with neutrophils and macrophages, producing active forms of oxygen [1], or when acted upon by chemotherapeutic agents capable of forming free radicals [9]. Oxidative action on mammalian cells is accompanied by considerable destructive changes in the plasma membrane, such as increased permeability for high-molecular-weight substances [8], disturbance of transmembrane ionic currents [2], and reduction of flowability [4]. We know that the structural and functional properties of biological membranes are maintained by a strictly definite composition of the phospholipids, and an excessive content of derivatives such as lysophospholipids leads to destabilization of the lipid bilayer of the membranes and, as a result, to death of the cell [11]. A possibility that lysophosphatidylcholine accumulates in membranes during their oxidation has been demonstrated [12]. Lysophospholipids are reduced to diacyl-glycerol phospholipids by specific acyl-CoA-lysophospholipid acyltransferases (ACLAT) [10]. It can therefore be postulated that oxidative cell damage will be accompanied by disturbance of repair processes in cell membranes and, in particular, of phospholipid reacylation.

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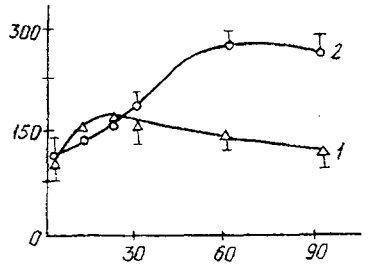

Fig. 1

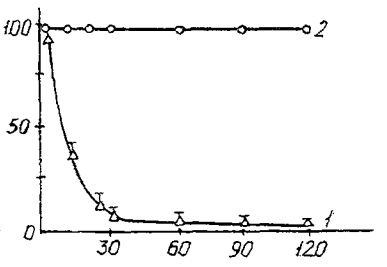

Fig. 2

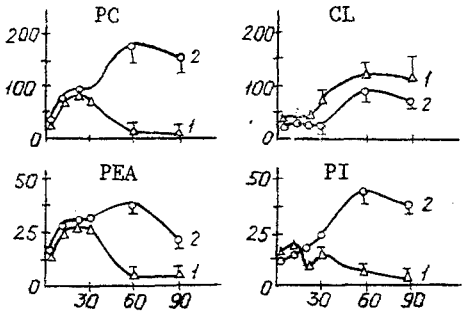

Fig. 3

Fig. 1. Inhibition of incorporation of [1-^{14}C]arachidonic acid into total phospholipids of P815 mastocytoma cells under the influence of *tert*-butyl hydroperoxides (1) relative to control (2). Abscissa, incubation time (in min); ordinate, incorporation of arachidonic acid (in cpm × $10^3/\mu$g inorganic phosphorus).

Fig. 2. Inhibition of incorporation of [1-^{14}C]arachidonic acid into P815 mouse mastocytoma cells under the influence of *tert*-butyl hydroperoxides (1) relative to control (2). Abscissa, incubation time (in min); ordinate, incorporation of arachidonic acid (in %).

Fig. 3. Inhibition of incorporation of [1-^{14}C]arachidonic acid into phospholipid fractions of P815 mastocytoma cells under the influence of *tert*-butyl hydroperoxides (1) relative to control (2). PC) Phosphatidylcholine, CL) cardiolipin, PI) phosphatidylinositol, PEA) phosphatidylethanolamine. Abscissa, incubation time (in min); ordinate, incorporation of arachidonic acid (in cpm × $10^3/\mu$g inorganic phosphorus).

The aim of this investigation was to study incorporation of [1-^{14}C]arachidonic acid (AA) in mouse mastocytoma P815 tumor cells and in different phospholipid classes under the influence of *tert*-butyl hydroperoxides (TBHP).

METHODS OF INVESTIGATION

Mastocytoma P815 cells, transplanted intraperitoneally into DBA2 mice were used in the experiments. Oxidation of the cells was carried out by TBHP in a concentration of 16 mM. Incorporation of [1-^{14}C]AA ("Amersham," England, specific activity 58.3 mCi/mmole) into isolated cells (0.2 μCi/1.5 · 10^6 mastocytoma cells) was carried out at 37°C for 5 min, and the excess of AA was removed by medium 199 with 0.1% albumin, the cell suspension being centrifuged for 5 min at 2000*g*. Calculation of the radioactive label in the cell residue was carried out on a Mark-3 liquid scintillation counter (The Netherlands). To determine incorporation of [1-^{14}C]AA into individual phospholipid classes, lipids were extracted from the cells by the method in [3]. Separation of phospholipids into classes was carried out by HPLC, using an "Ultrachrome GTi" chromatograph (LKB, Sweden), on an "Ultropac TSK-Si-150" column,

particle diameter 5 μ. The mobile phase consists of system 1: hexane:isopropanol (80:20, V/V) and system 2, namely isopropanol:hexane water (52:39:9, v/v). Elution began with 100% of system 1 for 5 min, followed by a linear gradient from 100% of system 1 to 100% of system 2 over a period of 30 min; rate of flow 1 ml/min. The eluted fractions were collected in scintillation flasks every 60 sec for measurement of radioactivity. An a aliquot of phospholipid extract was used during determination of the content of lipid phosphorus in the sample by the method in [14].

EXPERIMENTAL RESULTS

Incubation of cells of mouse mastocytoma P815 with arachidonic acid led to incorporation of exogenous M into cellular phospholipids; fatty acid was a major component of cardiolipin (42% of the radioactivity of total phospholipids), phosphatidylcholine (36%), and phosphatidylethanolamine (14.8%). This is partly in agreement with the results obtained in [15], which demonstrated previously considerable incorporation of [^{3}H]AA into choline-containing glycerophospholipids, phosphatidylinositol, and ethanolamine-containing glycerophospholipids of mastocytoma P815 cells. The temporary dependence of incorporation of [1-^{14}C]AA into phospholipids, which we observed (Fig. 1), may perhaps be evidence that the process of reacylation of phospholipids is involved in adaptation of cells to the conditions of culture in vitro.

Incubation of mouse P815 mastocytoma cells with TBHP for 60 min led to considerable inhibition of incorporation of [1-^{14}C]AA into them (Fig. 2); TBHP, moreover, had an inhibitory effect on the process of incorporation of arachidonic acid, both into total phospholipids (Fig. 1) and into phospholipid fractions, namely phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol (Fig. 3). After the end of the incubation period with TBHP the specific radioactivity of phosphatidylcholine was 15 times lower than that of the phosphatidylcholine of the control cells. For phosphatidylethanolamine and phosphatidylinositol, a 7- and 12-fold reduction of incorporation of the fatty acid respectively was observed. By contrast to this the content of [1-^{14}C]AA in cardiolipin of the experimental cells was rather higher than in the control. The different degree of inhibition of reacylation of phospholipids was evidently linked with the existence of ACLAT specific for each phospholipid class, and whose reaction velocity varies considerably [13]. We also know that processes such as inhibition of acyl-CoA synthetase, activation of acyl-CoA hydrolase, and a change in activity of phospholipases A_1 and A_2 may affect the velocity of phospholipid reacylation [10]. Another essential factor is the creation of an intracellular pool and provision for transport of fatty acids, for their interaction with proteins binding fatty acids. The possibility that the binding capacity of these proteins may be inhibited by substances generating O_2^- and $OH^\cdot$ has been demonstrated [7].

Inhibition of incorporation of arachidonic acid into cells and cellular phospholipids during oxidation may be a cause of the destabilization of cell membranes. Another factor to be taken into account is that deficiency of the supply of arachidonic acid, which is a precursor of biologically active compounds, may lead to a disturbance of the mechanisms of intracellular regulation. Evidence of the important role of arachidonic acid derivatives in the formation of the cellular response to damaging action has accumulated in recent years. For instance, exposure of cells to conditions of hyperoxia led to the accumulation of thromboxane B_2 and leukotrienes B_4 and C_4 in them [6]. Meanwhile prostaglandin E_1 prevented hyperoxic damage to alveocytes [5]. Inhibition of incorporation of [1-^{14}C]AA into P815 cells which we observed under the influence of TBHP may perhaps have led to a decrease in synthesis of biologically active compounds and, as a result, to a fall in the level of the cellular response to damaging action.

The process of enzymic reacylation of lysophospholipids thus participates in the repair of cell membranes, and its inhibition during oxidative injury may be one cause of death of tumor cells through the action of antracycline antibiotics and tumor-toxic macrophages and neutrophils.

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EFFECT OF DEFENSIN HNP-1 OF HUMAN NEUTROPHILS ON PRODUCTION OF TUMOR NECROSIS FACTOR α BY HUMAN BLOOD MONOCYTES IN VITRO

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KEY WORDS: defensins; tumor necrosis factor α; monocytes.

Intercellular interactions, including those between macrophages and granulocytes play an important role in the immune response. It has been shown that granulocytes can be activated by macrophagal cytokines: IL-8, IL-1, tumor necrosis factor a (TNF-α) [4, 13, 15]. Meanwhile, little is known of the effect of granulocytic soluble factors on monocytes/macrophages. One of the bactericidal mechanisms used by granulocytes is the system of the defensins and certain other proteins contained in granules of neutrophils [1, 9]. The defensins are a family of cationic peptides with mol, wt. of 3-4 kV, which account for 5-7% of the total cell protein in human neutrophils [8].

We have suggested that defensins, released from granulocytes into the extracellular median, can act on synthesis of macrophagal cytokines. The aim of our investigations was to study the effect of the defensin HNP-1 of human neutrophils on production of TNF-α in a culture of human monocytes.

EXPERIMENTAL METHOD

Mononuclear leukocytes (MNL) were isolated from fresh heparinized donated blood by the standard method [3]. Monocytes were obtained by fractionating MNL on preformed continuous Percoll density gradient ("Pharmacia") [10] at 4°C. The fraction of monocytes contained about 80% of cells giving a positive reaction for α-naphthyl acetate esterase (kit from "Sigma"). Monocytes ($2 \cdot 10^6$/ml) were cultured in medium RPMI 1640 ("Gibco") with antibiotics and L-glutamine in plastic Petri dishes ("Flow Laboratories"). Heat-inactivated fetal calf serum (FCS) (0.1%) was added to the monocyte cultures 30 min after the beginning of incubation. To induce TNF-α, a bacterial suspension of *Staph. aureus* Cowan 1 (SAC), prepared by the method in [11] was used, in a final concentration of 0.00025-0.001% (by volume) in the culture medium together with phorbol myristate acetate (PMA) ("Calblochem")

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