LOW ANTIOXIDANT ENZYME ACTIVITY IN TUMOR CELLS AS A FACTOR IN OXYGEN DEPENDENCE OF ANTITUMOR CYTOTOXICITY OF MACROPHAGES

E. V. Borunov, I. V. Khavalkin, L. P. Smirnova,
I. A. Shchepetkin, V. Z. Lankin, and N. V. Vasil'ev

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Tissue macrophages and their precursors (monocytes, promonocytes) are among the principal effector cells of the system of natural antitumor resistance of the body. Unlike normal killer cells, they can produce lysis not only of leukemic target cells, but also of target cells isolated from solid tumors, which are cytotoxic for autologous, allogeneic, and xenogeneic tumor cells [1, 7-9]. With the broadening of the spectrum of methods available for activating macrophages the number of cell lines resistant to macrophagal cytotoxicity has been reduced [2]. The universality of cytotoxicity may be the result of the diversity of the mechanisms of its realization. In fact, many soluble cytotoxic factors of macrophages have now been described: serine proteases, arginase, tumor-necrotizing factor, active forms of oxygen, lysosomal enzymes, lysozyme, and interferon [2, 3, 6]. Each of these factors evidently has a specific metabolic pathway or specific stages along the metabolic pathway of realization of cytotoxicity. Evidently each metabolic pathway can be modified by the target cell through limitation and repair of injuries. Consequently, the choice of pathways of realization of cytotoxicity depends not only on the presence of particular cytotoxic factors in macrophages, but also on the special features of metabolism of tumor target cells.

Because of the ability of macrophages to reduce molecular oxygen to the superoxide anion-radical and H_2O_2, it has been suggested [13] that one mechanism of injury of target cells by macrophages is through a process of membrane lipid peroxidation (LPO), induced by active forms of oxygen (AFO). Thus the participation of AFO-dependent mechanisms in cytotoxicity of macrophages will be determined both by their ability to produce AFO and by factors limiting "peroxide" membrane damage, including the level of activity of intracellular antioxidant enzyme.

EXPERIMENTAL METHOD

Analysis of correlation between the parameters of AFO production and cytotoxity of stimulated peritoneal macrophages of Wistar rats was carried out with the aid of inhibitors of the "respiratory burst" — namely preparations of the phenothiazine series: trifluoperazine (TFP), levomepromazine (LP), and chlorpromazine (CP). AFO production, revealed as chemiluminescence with luminol ("Sigma," USA), was recorded on the 1251 luminometer (LKB, Sweden) operating automatically. Membrane toxicity was determined from the release of ^{3}H-uridine from previously labeled target cells, with a ratio of effector to target of 1:10, followed by recording of the residual lable on a Mark 3 liquid scintillation counter ("Tracor Analytic," Holland). Enzyme activity was determined in a cell homogeneate after centrifugation at 4°C and 30,000g for 40 min. Catalase activity was measured spectrophotometrically as utilization of H_2O_2, glutathione peroxidase activity was measured in a coupled glutathione reductase system as oxidation of NADPH with tert-butyl hydroperoxide as the substrate, glutathione-S-transferase activity was determined from the formation of conjugates of 1-chloro-2,4-dinitrobenzine with glutathione, glutathione reductase activity was determine as oxidation of NADPH in the presence of oxidized glutathione, and glucose-6-phosphate dehydrogenase activity was determined as reduction of NADPH in the presence of glucose-6-phosphate. Mastocytoma strain P815, transplanted intraperitoneally in DBA/2 mice, and strain K562 of human erythromyeloleucosis, maintained in culture, were used as target cells. The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney nonparametric U test.

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TABLE 1. Inhibition by Phenothiazines of AFO Production and Membrane Toxicity of Macrophages against K562 and P815 Cells

Preparation	Concentration, μM	Inhibition, %		
		of AFO production	of membrane toxicity to K562	of membrane toxicity to P815
LM	0.1	47*	8	—2
	1	88*	22*	9
	10	98*	34*	—4
CP	0.1	12	16	—2
	1	48*	32*	—6
	10	99*	48*	17
TFP	0.1	2	6	9
	1	15	14	6
	10	97*	34*	13

Legend. Membrane toxicity of macrophages in control for K562 cells was 50%; for P815 cells it was 47%. *$p < 0.05$ compared with control.

EXPERIMENTAL RESULTS

The phenothiazines caused dose-dependent inhibition of the ability of macrophages to produce active forms of oxygen when activated by opsonized zymosan. According to their concentrations inhibiting AFO production by 50%, the phenothiazines could be arranged in the following order: LP (ID_{50} = 0.15 μM), CP (ID_{50} = 0.8 μM), TFP (ID_{50} = 2.2 μM). We now know that reduction of molecular oxygen to the superoxide anion-radical is effected by the NADPH-oxidase enzyme complex of the macrophagal plasma membrane during activation of the cell. Components of NADPH-oxidase are activated by protein kinase C, but regulation of its activity depends on Ca^{++} and is mediated by calmodulin [12]. The phenothiazines are calmodulin antagonists and protein kinase C inhibitors [14], and for that reason they are perhaps able to depress AFO production by macrophages; this effect is connected with intracellular processes and it does not suffer from the disadvantages of investigating oxygen dependence of cytotoxicity with the aid of AFO traps or AFO utilization enzymes, which do not penetrate into the zone of the cell junction between macrophage and target cell [10].

Incubation of peritoneal macrophages in medium with phenothiazines inhibited their membrane toxicity relative to K562 cells in proportion to the concentration of the inhibitors (Table 1), thus confirming the connection between damage to the target cell membrane and AFO production by macrophages. However, membrane toxicity of macrophages in relation to P815 cells was not inhibited by TFP, CP, or LP.

It can be postulated that membrane toxicity of macrophages relative to K562 and P815 cells differs with respect to oxygen dependence: P815 cells are resistant to AFO whereas injury to K562 cells is brought about mainly by AFO. Attention is drawn to the fact that without exposure to phenothiazines membrane toxicity relative to both types of cells was equal in degree: 50% for K562 and 47% for P815. Allowing for the results of inhibition, this is evidence of a difference in the mechanisms of realization of membrane toxicity in these cells.

This difference in the dependence of damage to K562 and P815 cells on production of active forms of oxygen by macrophages may be attributable to differences in the activity of their antioxidant enzymes, utilizing AFC and limiting "peroxide" damage to their cell structures. Activity of coupled glutathione-dependent enzymes utilizing hydroperoxides (glutathione peroxidase and glutathione reductase) is in fact higher in P815 cells than in K562 cells by 6.5 and 2.5 times respectively (Table 2). Activity of glucose-6-phosphate dehydrogenase in the two different cell lines did not differ significantly, whereas catalase and glutathione-S-transferase activity was 3 times higher in K562 cells than in P815 cells. The opposite relationship between activity of catalase, which metabolizes H_2O_2, and oxygen dependence of cytotoxicity indicates that the role of the catalase reaction in the defense of tumor cells against AFO, generated by macrophages, is not determinant. This conclusion is in agreement with data in [5, 13], the authors of which did not observe any increase in sensitivity of tumor cells when their catalase was inhibited, with respect either to enzymically generated H_2O_2 or to activated macrophages, whereas inhibition of bioregeneration of glutathione increased the sensitivity of the tumor cells to both damaging factors. The authors cited explain their results on the grounds that, first, glutathione peroxidase has 3 times greater affinity for H_2O_2 than catalase and, second, glutathione peroxidase can reduce lipid hydroperoxides, and this is probably more important for protection of the cell membrane against injury. Our own data, indicating a possible connection between low glutathione peroxidase and glutathione

TABLE 2. Activity of Antioxidant Enzymes in Human Erythromyeloleucosis K562 Cells and Murine Mastocytoma P815 Cells

Enzymes	K562 ($n=8$)	P815 ($n=6$)
Catalase, μmoles H_2O_2/min/mg protein	1,5	0,4*
Glucose-6-phosphate dehydrogenase, μmoles NADP/min/mg protein	$3,6 \cdot 10^{-3}$	$5,6 \cdot 10^{-3}$
Glutathione reductase, μmoles NADPH/min/mg protein	$8,3 \cdot 10^{-3}$	$21,1 \cdot 10^{-3}$*
Glutathione peroxidase, μmoles NADPH/min/mg protein	$9,7 \cdot 10^{-3}$	$63,7 \cdot 10^{-3}$*
Glutathione-S-transferase, μmoles NADPH/min/mg protein	$6,9 \cdot 10^{-2}$	$2,2 \cdot 10^{-2}$*

Legend. *$p < 0.01$, n) number of experiments.

reductase activity in the cells and the AFO-dependence of their damage by macrophages, confirm the important role of enzymic utilization of lipid peroxides in the defense of tumor cells against macrophagal cytotoxicity.

Glutathione-S-transferase can exhibit peroxidase activity, but since its isozymes are also involved in reactions for the detoxication of xenobiotic (peroxides, epoxides, aldehydes), synthesis of leukotrienes, and metabolism of steroid hormones [11], the ability of glutathione-S-transferase to form conjugates of glutathione with 1-chloro-2,4-dinitrobenzene, which we investigated, does not necessarily correspond to its lipid peroxidase activity.

The results are thus in harmony with the view that antioxidant enzymes and, in particular, glutathione peroxidase and glutathione reductase play an important role in maintaining the resistance of tumor cells to the cytotoxic action of macrophages, mediated through active forms of oxygen.

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