ONCOLOGY

Effect of Lactate on Functional Activity of Macrophages under Normal Conditions and during Tumor Growth

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Lactate modulated functional activity of macrophages from intact mice and animals with transplantable syngeneic hepatoma 22a. Sodium lactate in a concentration of 6.5 mM *in vitro* increased functional activity of resident peritoneal macrophages from intact mice. The growth of transplantable syngeneic hepatoma 22a was associated with the absence of correlation between nitrite synthesis by peritoneal macrophages and serum lactate concentration. Therefore, this agent cannot be considered as a systemic activator of macrophages.

Key Words: *macrophages; tumors; lactate; nitrites; superoxide anions*

Lactate is now considered as a mediator of tissue hypoxia. Inadequate blood supply to wounds and central tumor areas is accompanied by transition of cells to anaerobic metabolism and enhanced production of lactate (end product of glycolysis). The release of lactate reflects progression of hypoxia or tissue injury. Lactate stimulates production of angiogenic factors by macrophages and synthesis of collagen by fibroblasts [7] and serves as a mediator of inflammation. Macrophages migrating from the vascular bed to the inflammatory focus are exposed to hypoxia and secrete lactate.

Lactate concentration in the circulating blood increases during wound healing [10], sepsis [12], tumor growth [8], and physical exercise in sportsmen [2]. These data suggest that lactate produces a systemic effect. Little is known about immunoregulatory activity of lactate (*e.g.*, effect on macrophages). Lactate in physiological concentrations (4-16 mM) stimulates production of proinflammatory cytokines by human blood mononuclear cells [12] and synthesis of vascular endothelial growth factor by rabbit macrophages [6].

Here we studied the *in vitro* effect of lactate on functional activity of peritoneal macrophages from intact mice. The systemic effect of this substance on macrophages was evaluated in mice with tumors.

MATERIALS AND METHODS

Experiments were performed on male C3HA mice weighing 18-20 g and obtained from the Rappolovo nursery (Russian Academy of Medical Sciences). Peritoneal exudate cells (PEC) were isolated by peritoneal lavage. The cells obtained from 5 mice were pooled and incubated with RPMI 1640 medium containing 10% fetal serum and sodium lactate (Sigma) for 24 h. We studied spontaneous and LPS-induced production of nitrites, spontaneous and phorbol ester-induced generation of superoxide anions in the NBT reduction tes, 5'-nucleotidase activity, and pinocytosis of neutral red [1]. In a special series PEC were incubated with the supernatant of cultured hepatoma 22a cells in macrophage culture medium (ratio 1:1) or in the pre-

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sence of hepatoma cells (ratio 3:1). Cell viability was determined by trypan blue staining.

Live syngeneic hepatoma 22a cells (10^5 cells) were inoculated subcutaneously to study the effect of tumor growth. The hepatoma 22a cell line was presented by O. N. Pogodina (Institute of Cytology, Russian Academy of Sciences). Control animals received an equivalent volume of physiological saline. The animals were killed by cervical dislocation at fixed time intervals after inoculation of tumor cells. Serum lactate concentration was measured using Vital Diagnosics kits. The cell composition of PEC was determined in smears stained by the method of Romanovsky—Giemsa. The population of PEC included 30-40% macrophages, 60-65% lymphocytes, 1-3% mast cells, and solitary neutrophils.

The results were analyzed by Student's *t* test and pairwise correlation analysis.

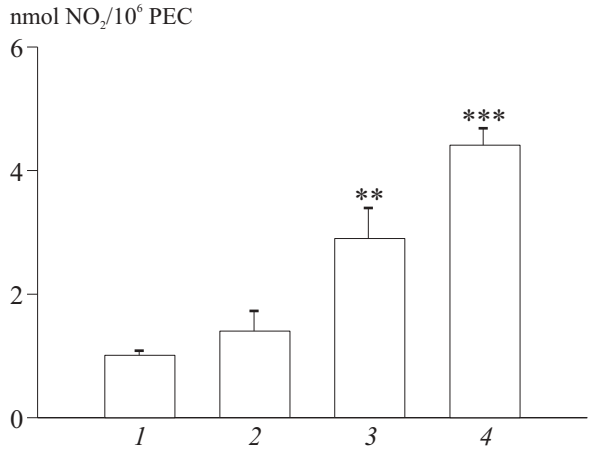

Fig. 1. Nitrite production by macrophages from intact mice under control conditions (*1*), in the presence of 100 ng/ml LPS (*2*), during coculturing with hepatoma 22a cells (*3*), and after addition of 48-h supernatant from cultured hepatoma cells. Here and in Fig. 2: **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the control.

RESULTS

Coculturing of macrophages from intact mice with hepatoma cells was accompanied by a 3-fold increase in nitrite production (Fig. 1). A similar effect was observed during coculturing of human blood monocytes with several lines of tumor cells [15]. It was interesting to evaluate whether the stimulatory effect develops after direct contact with hepatoma cells or is mediated by soluble factors. NO_2 production by macrophages from intact mice was studied in the presence of tumor cells cultured for 48 h. We revealed stimulation of nitrite production. Supernatants of cultured hepatoma 22a cells contained 10-13 mM lactate, but did not include nitrites. It could be hypothesized that lactate stimulates NO_2 production by macrophages. To test this hypothesis, nitrite production by peritoneal macrophages from intact mice was studied in the presence of lactate at a concentration corresponding to that in the diluted supernatant of hepatoma cells (6.5 mM, Table 1). The intensity of nitrite production during culturing of macrophages with lactate was much higher compared to that observed in the supernatant. It was probably due to the influence of other stimulating factors present in the supernatant of hepatoma cells.

Our results seem to contradict published data that lactate in a concentration of 25 mM has no effect on nitrite production by mouse peritoneal macrophages [14].

We also studied the effect of lactate on other functional characteristics of macrophages. The test compound increased spontaneous and induced production of superoxide anions, stimulated pinocytosis, and decreased 5'-nucleotidase activity. This phenotype is typical of the classically activated macrophage (Table 1). We showed for the first time that lactate *in vitro* modulates pinocytosis, superoxide anion generation, and 5'-nucleotidase activity in cells of the mononuclear-phagocytic system.

The role of lactate as systemic activator of mononuclear-phagocytic system cells was studied *in vivo*

TABLE 1. *In Vitro* Effect of Lactate on Functional Activity of Peritoneal Macrophages from Intact Mice (*M*±*m*)

Parameter		Control	Sodium lactate, 6.5 mM
NO_2^- production, nmol/10^6 PEC		0.43±0.02	0.71±0.05***
NBT test, OD_{620}	spontaneous	0.045±0.003	0.128±0.033*
	phorbol ester-induced	0.265±0.007	0.397±0.023***
Neutral red pinocytosis, OD_{540}		0.491±0.016	0.546±0.022*
5'-nucleotidase, nmol/10^6 PEC/h		49.86±0.86	39.17±3.39*
Cell viability, %		95.2	86.5

Note. PEC were pooled from 5 mice. Each experiment was performed in 2-3 repetitions. OD_{620} and OD_{540}: optical density at 620 and 540 nm, respectively. **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to the control.

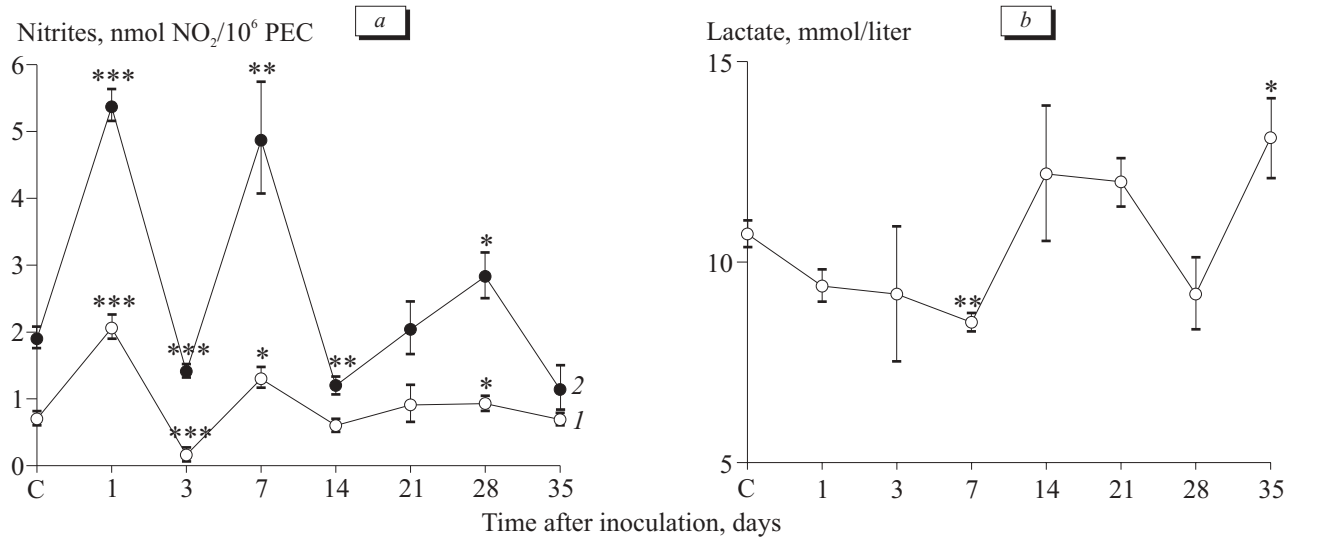

Fig. 2. Nitrite production (*1*, *2*) by peritoneal exudate cells (*a*) and serum lactate concentration during growth of hepatoma 22a (*b*). Control group, 40-50 mice; experimental group, 8-10 mice. C: control.

on mice with hepatoma. Nitrite synthesis by mouse peritoneal macrophages was studied during tumor growth. The results obtained in this series were compared with lactate concentration in the circulating blood. Production of nitroxide anions by mouse macrophages increased on days 1, 7, and 28 after inoculation of hepatoma cells (Fig. 2, *a*). Our results are consistent with published data that the growth of experimental tumors is accompanied by stimulation of nitrite production by macrophages [5].

Serum lactate concentration in control C3HA mice was 10.7±0.3 mmol/liter (n=42). Blood lactate concentration significantly decreased on day 7, but increased on day 35 after inoculation of tumor cells (Fig. 2, *a*). The increase in blood lactate concentration in the late stage of tumor growth was observed in previous experiments on rats [8].

A negative correlation was found between lactate concentration and production of nitroxide anions (insignificant). Therefore, the increase in functional activity of macrophages during hepatoma growth did not depend on lactate concentration in circulating blood. Activation of macrophages is probably associated with the influence of other metabolites or products of tumor cells.

It was hypothesized that lactate act as a systemic activator of macrophages during tumor growth [13]. Malignant cells can secrete considerable amounts of lactate due to specific features of metabolism [4]. However, lactate is rapidly consumed by tissues during gluconeogenesis to maintain normoglycemia [3]. It should be emphasized that tumor cells not only produce, but also utilize lactate under conditions of its increased blood concentration [11]. Macrophages can utilize lactate through specific transport proton systems [9]. Lactate concentration increases insignificantly at the late stage of solid tumor growth in humans and animals, which agrees with our findings. Probably, blood lactate concentration should increase more significantly to produce the systemic immunoregulatory effect. These changes are usually observed in patients with sepsis and under conditions of vigorous physical exercise in sportsmen.

Our results indicate that during tumor growth lactate acts as a local mediator, but does not produce systemic effects. These findings complement the data that lactate stimulates production of various cytokines by macrophages (*e.g.*, tumor necrosis factor-α, interleukin-1β, interleukin-6, and vascular endothelial growth factor) [6,12, 14]. Activated macrophages can increase not only tumoricidal activity, but also proangiogenic function. Further investigations are required to estimate the effect of lactate on macrophages in tumor nodes.

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