ONCOLOGY

Effect of Plasma Lipoproteins and Their Complexes with Polysaccharides on Interleukin-1β Concentration in Macrophages of Mice with HA-1 Ascitic Hepatoma

L. M. Polyakov, D. V. Sumenkova, and L. E. Panin

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Experiments on cultured peritoneal macrophage from mice with HA-1 ascitic hepatoma showed that plasma lipoproteins present in the incubation medium decreased intracellular concentration of interleukin-1β. These changes were most pronounced for high-density lipoproteins (alone or in combination with cortisol). Bacterial and yeast polysaccharides had little effect on interleukin-1β concentration in macrophages. Addition of polysaccharides in combination with lipoproteins was followed by a 2-3-fold decrease in interleukin-1β concentration. A combination of polysaccharides and high-density lipoproteins had the strongest effect. These properties of plasma lipoproteins should be taken into account in the correction of macrophage function during tumor growth.

Key Words: *macrophages; interleukin-1β; lipoproteins; polysaccharides; HA-1 hepatoma*

Macrophages play an important role in antitumor protection [10]. Cytotoxic activity of these cells is associated with secretion of some cytokines and reactive oxygen metabolites [13,14]. However, recent studies showed that macrophages are involved in tumor progression. Functional activity of macrophages are modulated by tumor microenvironment: these cells gain the ability to maintain tumor growth, which is realized via stimulation angiogenic activity and increase in the invasive and metastatic potential of the tumor [10]. Proinflammatory cytokine interleukin-1β (IL-1β) is a regulatory macrophage factor. IL-1β promotes tissue regeneration and stimulates metastatic dissemination during tumor growth [6]. Some attempts were made to neutralize IL-1β in oncological diseases. However, many drugs do not satisfy the demands. In light of this, the search for new products that circulate for a long time and provide a strong therapeutic effect is an important problem. We believe that these properties are typical of plasma lipoproteins.

Chronic inflammation plays an important role in tumor development [4]. Various polysaccharides, including bacterial lipopolysaccharides (LPS) and yeast carboxymethylated (CMG) or sulfoethylated glycans (SEG), can induce chronic inflammation.

Here we studied the effects of high-density lipoproteins (HDL), low-density lipoproteins (LDL), and their complexes with polysaccharides on IL-1β concentration in peritoneal macrophages from mice with HA-1 ascitic hepatoma.

MATERIALS AND METHODS

Experiments were performed on peritoneal macrophages from male A/Sn(A) mice aging 3-4 months

Institute of Biochemistry, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** plm@soramn.ru. L. M. Polyakov

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and weighing 20-24 g. HA-1 ascitic hepatoma was induced by *o*-aminoazotoluene (Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk). Tumor-associated macrophages were isolated from the ascitic fluid due to adhesive properties of cells. Ascitic fluid cells were resuspended in RPMI-1640 medium with 2 mM L-glutamine (Biolot, pH 7.4) containing 20 mM HEPES (ICN Biomedicals, Inc). The cells were incubated in 6-well plates (Orange Scientific) in a CO_2 incubator (5% CO_2 and 95% O_2; Cole-Parmer) at 37°C. After 30-min incubation, the cell monolayer was washed 2 times with RPMI-1640 medium to remove nonadherent cells. Further incubation of tumor-associated macrophages was performed as described above. The density of cultured cells was 1500 cells/mm^2.

Plasma lipoproteins were isolated by isodensity ultracentrifugation in KBr solutions in the presence of 3 mM EDTA-Na_2. Ultracentrifugation was performed in an Optima L-90K centrifuge (Beckman Coulter) equipped with an angle rotor (type 70.1 Ti) at 105,000*g* for 24 h. Lipoproteins were dialyzed against 0.05 M potassium phosphate buffer (pH 7.4) containing 0.15 M NaCl at 4°C for 24 h. Lipoproteins were added to the macrophage incubation medium with a protein concentration of 1 mg/ml.

Experiments were performed with bacterial polysaccharides (*E. coli* LPS, Sigma) and yeast polysaccharides (CMG and SEG; Institute of Chemistry, Slovakian Academy of Sciences). Polysaccharides in a concentration of 10 μg/ml were added to the macrophage incubation medium. After 2 h, the cells were lysed with a solution containing 10 mM sodium phosphate (pH 7.2), 85 mM NaCl, 5 mM KCl, 0.5% sodium deoxycholate, and 1% Triton X-100.

IL-1β concentration in the cell lysate was measured by solid-phase enzyme immunoassay using a ProCon IL1-beta commercial test system. The measurements were performed on a Multiscan MCC-340 vertical photometer at 450 nm. IL-1β concentration in control samples was 43.3±3.7 pg/ml.

The results were analyzed by Student's *t* test at a significance level of $p<0.05$.

RESULTS

Table 1 shows that 2-h incubation of mouse peritoneal macrophages with HA-1 ascitic hepatoma in the presence of lipoproteins was followed by a decrease in intracellular IL-1β concentration. These changes were most pronounced in the presence of HDL. Under these conditions, IL-1β concentration decreased by 8 times compared to the control. The presence of LDL in the incubation medium was accompanied by a 2-fold decrease in IL-1β concentration. Glucocorticoids or their synthetic analogues are extensively used as inductors of tumor cell apoptosis in the therapy of tumors [2]. Moreover, glucocorticoids can inhibit the production of proinflammatory cytokines by macrophages [8]. In our experiments, the effect of cortisol (10^{-6} M) was similar to that of HDL. IL-1β concentration decreased by 9.6 times compared to the control (4.5±0.9 pg/ml, $p<0.05$). Combined treatment of macrophages with HDL and cortisol induced a greater decrease in IL-1β concentration (by 19 times). Our results are consistent with published data [9].

Polysaccharides are involved in the inflammatory response, which plays the major role at various stages of tumor formation. The adverse effect of these polysaccharides is mediated by secretory products of macrophages, including tumor necrosis factor, IL-1, and IL-6 [5,12]. They play an important role in various stages of tumor formation, including the initiation, promotion, and progression [11]. LPS-binding protein [15] and lipoproteins [1,3] have a particular role in LPS binding in blood plasma. It was hypothesized that lipoprotein-bound LPS cannot induce macrophage activation [7].

We showed that addition of polysaccharides in combination with lipoproteins is followed by a decrease in IL-1β concentration by 1.5-3.0 times compared to the control (Table 1). A combination of polysaccharides and HDL had the strongest effect. Individual treatment with polysaccharides had no effect on IL-1β concentration in macrophages. Probably, tumor-associated macrophages are primed and cannot induce further increase in the secretion of proinflammatory cytokine.

Our findings indicate that lipoproteins can produce a proinflammatory effect. The mechanism for the action of lipoproteins on functional activity of tumor-associated macrophages remains unknown. The effect of lipoproteins is probably related to regulatory properties of their protein components (apolipoproteins). The formation of polysaccha-

TABLE 1. IL-1β Concentration in Peritoneal Macrophages from Mice with HA-1 Ascitic Hepatoma (pg/ml, *M*±*m*)

Additives in the incubation medium	HDL	LDL
No additives	5.3±1.0*	20.2±2.3*
Cortisol	2.3±0.4*	15.4±1.2*
LPS	12.8±1.8*	25.8±2.6*
CMG	16.1±1.0*	30.1±2.8*
SEG	13.5±0.7*	28.0±1.5*

Note. *$p<0.05$ compared to the control.

ride-lipoproteins complexes in blood plasma serves as a defense mechanism, which reduces the severity of chronic inflammation during tumor growth.

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