Stimulation of Macrophages Increases, While Suppression of These Cells Inhibits Metastatic Dissemination of Two Transplantable Mouse Tumors in the Liver and Lungs

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Stimulation of mouse tissue macrophages with carboxymethylated β-(1→3)-D-glycan 1 day before intravenous injection of tumor cells increased the number and weight of implants (experimental metastases) of mouse hepatocarcinoma and adenocarcinoma in the liver and lungs, respectively. Suppression of liver macrophages with gadolinium chloride or sequestration of cells during intraperitoneal administration of macrophage attractants inhibited metastatic dissemination of hepatocarcinoma and adenocarcinoma in the liver and lungs, respectively. In the latter case animal lifespan increased. Our results indicate that at certain stages of metastatic dissemination, activation of mononuclear phagocytes can stimulate the formation and growth of metastases.

Key Words: *transplantable mouse tumors; metastatic dissemination; lungs; liver*

Mononuclear phagocytes play an important role in antitumor resistance [2,4,10,11]. Administration of exogenous macrophage inhibitors accelerated tumor growth. It was hypothesized that bioactive substances, macrophage stimulators, and immunostimulators hold promise for preventing growth and metastatic dissemination of tumors [3,5,15]. Previous experiments showed that macrophages and immune system play an ambivalent role in the regulation of tumor growth [6, 13,14]. There are contradictory data on their effect on metastatic dissemination. Some authors reported that stimulation of macrophages inhibits metastatic dissemination [5,15]. Other investigators showed that stimulation of macrophages increases metastatic dissemination: increases the number of metastatic nodes and accelerates tumor growth [4,6,11]. Suppression of macrophages produces different effects on metastatic dissemination of tumors [2-4,11]. Morphometry showed that suppression of liver macrophages does not stimulate, but inhibits the formation of experimental metastases from mouse hepatocarcinoma (HA-1) in the liver [1].

Here we studied the role of tissue macrophages in metastatic dissemination of transplantable mouse tumors HA-1 and lung adenocarcinoma (LA). Experiments were performed with inhibitors and stimulators of macrophages. These substances modulate macrophage function during colonization of organs that serve as a target for metastatic dissemination of tumor cells circulating in the vascular bed.

MATERIALS AND METHODS

Experiments were performed on adult male A/Sn mice obtained from the vivarium of the Institute of Cyto-

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logy and Genetics. The animals were kept in cages (6-8 specimens per cage) under the natural light/dark regimen and had *ad libitum* supply of granulated complete fodder (PK 120-1, Laboratorsnab) and water. Experimental metastases were produced by administration of HA-1 (1×10^5 or 0.5×10^5) and LA cells (3×10^4) into the lateral caudal vein. Macrophage function in target organs for metastatic dissemination was suppressed with gadolinium chloride ($GdCl_3$), dextran sulfate (500 kDa, inhibiting fusion of phagosomes with lysosomes [12]) or by intraperitoneal injection of macrophage attractants (starch or colloidal iron, 0.4 ml 10% solution). Carboxymethylated β-(1→3)-D-glycan (CMG; Institute of Chemistry, Slovakian Academy of Sciences) served as macrophage stimulator. Macrophage inhibitors and stimulator were injected intravenously 1 day before tumor transplantation ($GdCl_3$ and dextran sulfate, 10 mg/kg; CMG, 25 mg/kg). Control animals received no injections.

The mice with LA were decapitated 20 days after tumor cell transplantation. The lungs were fixed with 10% formalin. Lung metastases were counted under a binocular loupe. The animals with HA-1 were oberved until death. We estimated the length of animal survival after tumor cell transplantation. The mice were killed, and body weight and weight of the liver were measured. The average daily increase in tumor weight was calculated as follows: (ILt-ILi)/(P×1000), where ILt is the index of liver function in mice with tumors (ratio between the weight of the liver and body weight); ILi is the index of liver function in intact mice of the same strain, sex, and age; P, is body weight of mice with tumors (in grams); and 1000 is the factor for conversion into milligrams.

The results were analyzed by nonparametric Wilcoxon—Mann—Whitney test and Student's *t* test.

RESULTS

Control mice receiving 1×10^5 HA-1 died from massive tumor implants in the liver (experimental metastases) 25.00±1.16 days after treatment. CMG had no effect on animal lifespan and mean daily increase in tumor weight (Table 1). $GdCl_3$ decreased the rate of tumor growth in the liver (by 25% compared to the control) and increased mouse lifespan (Table 1).

The mice implanted with a 2-fold lower number of HA-1 cells (0.5×10^5) also died from liver damage. However, the lifespan of these animals was higher compared to mice receiving 1×10^5 HA-1 cells (by one third time). Under these conditions $GdCl_3$ significantly increased the lifespan of mice with tumors. It should be emphasized that CMG increased the weight of tumor implants in the liver and, therefore, stimulated metastatic dissemination (Table 1).

This experimental model did not allow us to perform quantitative analysis of confluent metastases due to significant number of these metastases. This problem was solved in further experiments on the model of LA. After intravenous injection, LA cells are implanted in the lungs and produce a limited number of metastases. In mice transplanted with tumor cells after administration of CMG, the number of lung metastases was 2-fold higher compared to the control (Table 2). Published data show that $GdCl_3$ at a specified concentration suppresses macrophage function only in the liver [2,9]. To suppress function of lung macrophages, they were mobilized into the abdominal cavity

TABLE 1. Effects of Macrophage Stimulator and Inhibitors on the Number and Growth Rate of Experimental Metastases from HA-1 in Mouse Liver after Intravenous Transplantation of Tumor Cells

Tumor, group	Number of mice	Lifespan, days	Tumor weight, mg	Mean daily increase in tumor weight, mg	Ascites, g
HA-1 (100,000 cells intravenously)					
control	13	25.00±1.16	2052±171	85.0±6.8	
dextran-sulfate	12	24.00±0.84	1693±110	74.0±6.5	
GdCl3	13	27.50±1.85*	1671±155	64.0±8.5*	
CMG	13	25.20±1.24	2037±128	82.0±6.2	
HA-1 (50,000 cells intravenously)					
control	18	32.70±1.49	3135±248	100.0±7.5	1.90±0.74
dextran-sulfate	16	31.50±1.65	3117±235	104.0±9.2	0.52±0.25*
GdCl3	15	37.70±1.51*	3745±315	101.0±8.5	3.32±0.55
CMG	17	34.7±1.49	4210±350	124.0±10.6*	3.44±0.55

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

TABLE 2. Effects of Macrophage Stimulator and Inhibitors on the Number and Growth Rate of Experimental Metastases from LA in Mouse Lungs after Intravenous Transplantation of Tumor Cells

Group	Number of mice	Number of metastases per mouse	Mean weight of the lungs, mg
Control	19	48.0±5.5	340±15
Gelatin	12	24.0±3.9**	—
GdCl3	10	37.0±9.7*	335±15
CMG	10	93.0±13.3**	381±20

Note. *p<0.05 and **p<0.01 compared to the control.

by treatment with colloidal iron. The number of lung metastases in mice receiving gelatin 30 min before tumor cell transplantation was 2-fold lower than in animals of the control group (Table 2). Intraperitoneal administration of another macrophage attractant starch tended to decrease the weight of metastatic nodes in the liver of mice with HA-1 (Table 1).

Dextran sulfate had little effect on metastatic dissemination of HA-1 in the liver, but prevented abdominal ascites in mice with tumors (Table 1). Probably, the delayed consequences of treatment with dextran sulfate include membrane stabilization and decrease in vascular permeability. These changes can be associated with inhibition of hyaluronidase.

Our results indicate that stimulation of macrophages contributes to metastatic dissemination of LA cells in mouse lungs and slightly increases the weight of HA-1 metastases in the liver. Suppression of macrophages or decrease in cell count was followed by reduction of the number and weight of metastases from LA and HA-1 in the lungs and liver, respectively. These data show that stimulation of tissue macrophages increases the number of implanted tumor cells in the organ, while suppression of cell function produces an opposite change. Macrophages contain considerable amounts of proteolytic enzymes and are probably recruited by tumor cells for destruction of the extracellular matrix in target organs for metastatic dissemination. These changes occur during fusion of tumor cells with macrophages under conditions of partial phagocytosis [6]. Published data show that macrophages are involved in paracrine stimulation of tumor cell growth and angiogenesis in metastatic nodes [11]. After treatment with $GdCl_3$ gadolinium is accumulated in lysosomes and decrease the ability of macrophages to perform receptor-mediated phagocytosis [2,9]. These changes are probably followed by a decrease in the number of circulating tumor cells engulfed by macrophages. Our findings indicate that under certain conditions or at certain stages of metastatic dissemination, activation of mononuclear phagocytes can stimulate the formation and growth of metastases.

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