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INCREASED PRODUCTION OF TUMOR NECROSIS FACTOR DURING ENDOTOXIN SHOCK IN MICE PRESENSITIZED WITH SERUM OF MICE WITH TUMORS OR WITH TUMOR CELL FACTORS

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Experimental treatment of tumors based on the use of components of bacterial cells and, in particular, bacterial glycoconjugates, is currently regarded as highly effective [2, 6, 11, 12]. The main obstacle to the use of components of bacterial cell walls in clinical oncology is their toxicity. We know that animals with tumors are most sensitive to the toxic action of immunomodulators of bacterial origin [3, 4].

An increase in the sensitivity of animals with growing tumors to the toxic action of endotoxin correlates with the granulocytosis [3] and enhancement of the bactericidal activity of macrophages [4], and the monocytes of cancer patients can produce an increased quantity of tumor necrosis factor (TNF) and prostaglandin E_2 in response to stimulation by lipopolysaccharide [10]. The authors cited consider that the increase in sensitivity of tumor-bearing animals to the toxic action of lipopolysaccharide (LPS) is linked with activation of macrophages and is effected through increased production of cytokines (TNF etc.) by cells of the reticuloendothelial system.

The aim of this investigation was to study what factors are responsible for sensitizing an animal with tumor cells to the toxic action of a combination of LPS and glucosaminylmuramyldipeptide (GMDP), and to shed light on the causes of the increased mortality of tumor-bearing animals following administration of bacterial immunomodulators.

EXPERIMENTAL METHOD

Mice of strains C57BL/6 (H-2^b), DBA/2 (H-2*), BALB/c (H-2*), A/Snell (H-2*), and (CBA × C57BL/6)F_1 hybrids (H-2*) of both sexes, aged 2-3 months, were obtained from the "Stolbovaya" and "Svetlye Gory" nurseries Syngeneic tumor cells were injected into the mice: leukemia EL-4, melanoma B16, mastocytoma P815, and plasmacytoma MOPC 315, subcutaneously (10^6 cells).

*Haplotypes omitted in original – Translator.

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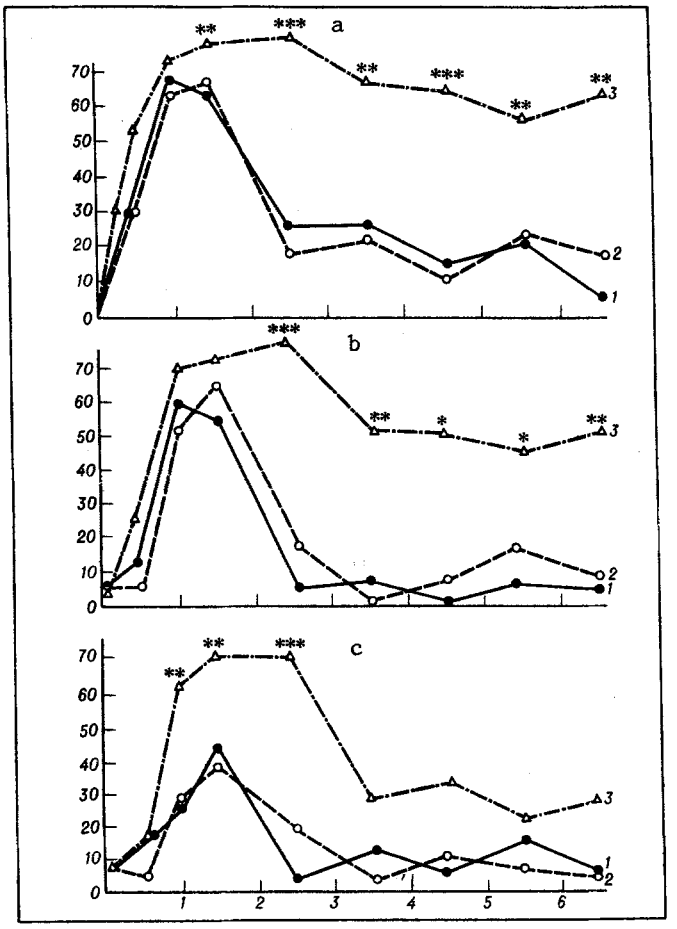

Fig. 1. TNF activity in serum of BALB/c mice in response to injection of LPS and GMDP. Abscissa, time after injection of LPS and GMDP (in h); ordinate, cytotoxicity index (in %); a, b, c) dilutions of test serum 1/20, 1/100, and 1/400 respectively. 24 h Before injection of LPS and GMDP mice received: 1) PBS, 2) serum of intact C57BL/6 mice, 3) serum of C57BL/6 mice with subcutaneous EL-4 tumor. *p > 0.02, **p > 0.01, ***p > 0.001.

Serum and ascites fluid were obtained from the intact animals or animals with subcutaneous tumors (on the 12th day of tumor growth). The ascites fluid was centrifuged at 1000 rpm for 5-7 min and the supernatant was collected and kept at −20°C The technique used to obtain cultural supernatants was described by the writers previously [9].

We showed previously that a combination of LPS and GMDP causes more intensive production of TNF by mouse splenocytes than each preparation separately [1], and for that reason, in experiments to study activation of TNF production in vivo, we used a combination of these preparations. Serum was obtained from the mice at various times after injection of the immunomodulators, and tested for its content of TNF by Fisch's method [8].

Sera from tumor-bearing mice were filtered through filters with different pore diameter (IM2, PM10, PM30, and XM50, allowing passage of substances with molecular weight of under 1, 10, 30, and 50 kD respectively) on a Diaflo apparatus (Amicon). The serum fractions were tested for their ability to induce sensitization to endotoxin shock.

In all the experiments the material tested for ability to induce sensitization (mouse sera, ascites fluids, etc.) were injected intravenously into the retro-orbital sinus in a volume of 0.2 ml. The mice were given an intravenous injection of LPS (20 μg) and GMDP (20 μg) in 0.2 ml PBS, 24 h after injection of the test materials. Mortality was assessed after 24 h.

TABLE 1. Effect of Sera from Tumor-Bearing Mice on Sensitivity of Intact Mice to the Toxic Action of a Combination of LPS and GMDP

Recipient mice	Control (no injection)	Control (injection)	Serum of C57BL/6 mice			Serum of DBA/2 mice		Serum of BALB/6 mice	
			intact	with BL-4 tumor	with B16 tumor	intact	with tumor P815	intact	with tumor MOPC 315
BALB/c	1/5 (20)	3/15 (14)	1/10 (10)	10/10 (100)	ND	ND	ND	0/6 (0)	10/10 (100)
C57BL/6	ND	0/5 (0)	1/5 (20)	5/5 (100)	ND	ND	ND	ND	ND
DBA/2	ND	0/5 (0)	0/5 (0)	5/5 (100)	ND	ND	ND	ND	ND
(CBA×C57BL/6F$_1$	1/5 (20)	0/5 (0)	0/5 (0)		4/5 (80)	0/5 (0)	3/5 (60)	ND	ND
A/Snell	0/5 (0)	0/5 (0)	0/5 (0)	10/10 (100)	ND	ND	ND	ND	ND

Legend. Numerator gives number of dying animals; denominator number of animals in experiment. Mortality (in %) in parentheses. ND) Not done.

The possibility of preventing sensitization was tested on models of tumor-bearing mice and mice sensitized with ascites fluids from mice with tumors. To protect against sensitization, indomethacin was injected intraperitoneally (100 μg/ml) in a volume of 0.5 ml. As tumor-bearers we used BALB/c mice with a subcutaneous MOPC 315 tumor. Indomethacin was injected twice: 24 h and 1 h before injection of LPS and GMDP. As the 2nd model we used A/Snell mice, sensitized by ascites fluid from C57BL/6 mice with a subcutaneous EL-4 tumor. Indomethacin was injected twice: 1 h before and 24 h after intravenous injection of ascites fluid (1 h before injection of LFS and GMDP).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that TNF production in response to injection of a combination of LPS and GMDP into the mice was considerably increased in mice receiving an injection of serum from tumor-bearing animals. Activity of TNF in the group receiving serum from tumor-bearing animals was observed at a high level for a sufficiently long time, up to 6.5 h after injection of the LPS and GMDP into the mice, whereas in the control (after injection of intact serum or PBS) activity was not observed 3 h after injection of LPS and GMDP. It was shown that TNF is in fact one of the main mediators of mortality from endotoxin shock [5].

It was stated previously that monocytes of cancer patients can produce increased amounts of TNF in response to stimulation by LPS [10], and also that during tumor growth activation of macrophages takes place [4]. It can be tentatively suggested that the serum of patients with tumors contains substances activating cells of the reticuloendothelial system, and this causes increased production of TNF under the influence of a combination of LPS and GMDP. The sensitizing factors in this case may perhaps be products of activated macrophages. Glucan, an activator of the RES, is known to increase sensitivity to endotoxin shock, whereas methylpalmitate, an inhibitor of RES, can protect animals against the toxic action of endotoxin [7]. It follows from the data in Table 1 that sensitivity to endotoxin shock can be transferred to intact mice by intravenous injection of small doses of serum from tumor-bearing mice. This rule is sufficiently universal in character, for it was successfully reproduced in five strains of mice with various syngeneic tumors.

We postulated that the actual products of the tumor cells may have a sensitizing action. The recipients were given an injection of serum from tumor-bearing animals or cultural supernatants of tumors EL-4 and B16, subcultured in vitro. It was found that for increased sensitivity to endotoxin shock to develop after injection of the cultural supernatants, prolonged contact (48 h) was necessary between the host and the factors contained in these supernatants. Meanwhile, for sensitization to develop after injection of serum from tumor-bearing animals 24 h was sufficient. This fact is indirect evidence that serum from tumor-bearing animals contains additional factors potentiating sensitization.

Intraperitoneal injection of indomethacin somewhat weakened the sensitizing effects both of the tumor itself and of factors contained in the peritoneal fluid of the tumor-bearing animals (Fig. 2). The results suggest that prostaglandins may be involved in the development of sensitization.

We obtained preliminary results relating to the problem of which fractions of the serum of tumor-bearing mice induce sensitization. In response to injection of serum of C57BL/6 mice, with a subcutaneous EL-4 tumor, into intact C57BL/6 mice, whole serum and fractions with mol. wt. of over 50 kD caused marked sensitization (Fig. 3).

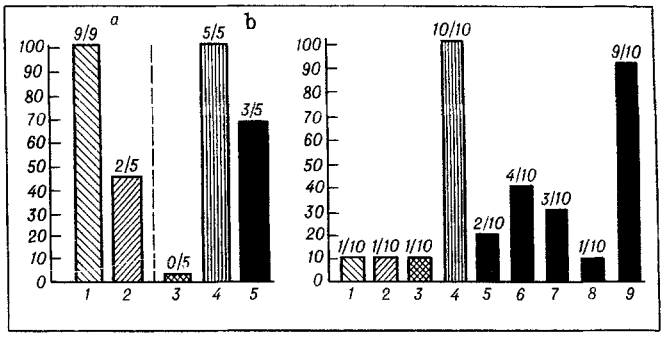

Fig. 2 Fig. 3

Fig. 2. Effect of indomethacin on tumor sensitization to the toxic action of LPS and GMDP. Ordinate, mortality (in %) a) BALB/c mice with subcutaneous MOPC 315 tumor: 1) without injection of indomethacin, 2) with injection of indomethacin; b) A/Snell mice: 3) intact, 4) with intravenous injection of ascites fluid from C57BL/6 mice with subcutaneous tumor EL-4, 5) with injection of ascites fluid from C57BL/6 mouse with subcutaneous tumor EL-4 and receiving indomethacin. Here and in Fig. 3: numbers above columns in numerator indicate number of dying animals, in denominator — number of animals in experiment.

Fig. 3. Effect of serum fractions from mice with tumors on sensitivity of intact mice to toxic action of LPS and GMDP. Ordinate, mortality (in %). 24 h Before injection of LPS and GMDP into C57BL/6 mice the following injections were given: 1) no injection (intact mice), 2) PBS, 3) serum of intact C57BL/6 mice, 4) whole serum of C57BL/6 mice with subcutaneous tumors EL-4 for fraction of this serum with one of the following molecular weights: 5) 1 kD, 6) 1-10 kD, 7) 10-30 kD, 8) 30-50 kD, 9) >50 kD.

We thus showed that the state of sensitization of tumor-bearing animals to endotoxin shock can be formed and transferred into intact animals by means of high-molecular-weight substances contained in the serum of the tumor-bearing animals, and which are evidently products of interaction of the host's cells and tumor cell factors. The state of sensitization is accompanied by hyperproduction and prolonged persistence of TNF in the serum of the sensitized animals under the influence of a combination of LPS and GMDP, and it is partly determined by increased prostaglandin production.

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