ONCOLOGY

Effect of Prostaglandin E_2 on the Chemiluminescence Activity of Peritoneal Exudate Cells of Intact Animals and Animals Inoculated with Different Materials

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As shown in the experiments *in vivo*, intraperitoneal administration of certain biological materials may induce stimulation and activation of the cytotoxic activity (CTA) of peritoneal exudate cells (PEC) [3,6,9]. The cytotoxic activity of PEC (macrophages, neutrophils, and lymphocytes) is apparently the first stage of protection of the organism against "foreign material", including bacteria and cells entering the peritoneal cavity. The reaction of PEC to administration of various materials may differ significantly and is manifested, in particular, in changes in the cell composition of the peritoneal exudate. These changes appear as early as the first hours after injection. However, neither the changes themselves nor the possibilities of their correction have been sufficiently studied. Prostaglandins of type E with a well-expressed immunodepressive activity with respect to lymphocytes (T and NK), together with neutrophils and, to a lesser extent, macrophages belong to the CTA inhibitors of PEC [2,5,8].

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The object of the present study was to investigate 1) the changes in the morphological composition of PEC and their chemiluminescent (CL) activity during the first hours after administration of different agents and 2) the effect of PGE_2 on the CL activity of intact and stimulated PEC.

MATERIALS AND METHODS

For intraperitoneal inoculation of the animals we used the following materials: 3% solution of thioglycolate prepared on Hanks buffer (dose 5 ml/hamster); 0.2% casein in Hanks buffer (dose 4 ml/hamster), mineral oil (dose 1 ml/hamster) and BCG vaccine, freeze-dried, suspended in Eagle's medium in a concentration of 3-5 mln microbial bodies per ml (dose 1 ml/hamster).

Syrian hamsters (2-3-months old) were used as the laboratory animals. Peritoneal exudate cells were collected 3-5 h after intraperitoneal administration of the agents. The peritoneal cavity of the animals was washed with 20 ml of culture medium containing hydrolysate of lactalbumin, 5% calf serum, 100 IU/ml monomycin, and 4-10 IU/ml heparin. The exudate cells were washed twice by centrifugation at

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TABLE 1. Changes in Normal Cellular Content of Peritoneal Exudate of Syrian Hamster after Intraperitoneal Administration of Different Materials and Suppression of CL Reaction of PEC by Prostaglandins *in Vitro* ($M \pm m$)

Material	Cellular composition of PEC, %			PGE_2, M	CL, × 10 cpm	
	M	L	N		PCL	SCL
Intact PEC	65.3±4.5	29.1±6.6	5.6±2.9	–	11.5±28.9	1.7±2.7
				$10^{-5}-10^{-14}$	12.3±29.4	1.4±3.0
Thioglycolate	6.0±1.6	3.0±0.8	91.0±2.4	–	147.5±306.9	57.5±116.1
				$10^{-5}-10^{-9}$	61.2±151.7	24.0±56.7
Casein	2.0±2.3	1.8±0.6	96.3±2.8	–	40.8±138.5	5.7±13.4
				$10^{-5}-10^{-10}$	47.1±147.2	3.9±21.8
Mineral oil	7.2±1.1	10.6±6.2	81.2±8.3	–	58.9±174.0	6.2±23.5
BCG	3.3±1.2	2.3±0.7	94.3±1.3	–	90.1±226.6	10.5±20.6
				$10^{-5}-10^{-13}$	38.7±102.1	3.8±11.4

1000 g in a heparin-free medium for 7 min. The PEC pellet was suspended in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum, HEPES, and 100 IU/ml monomycin (medium A). The content of macrophages (M), neutrophils (N), and lymphocytes (L) was assessed in 1 ml of medium and their ratio was determined.

A commercial PGE_2 preparation (Sigma, USA) was dissolved in medium A and added to PEC samples in a concentration of 10^{-5}-10^{-14} M. After a 30-min incubation at 37°C the liberation of active forms of oxigen by intact and PGE_2-stimulated PEC was assessed from luminol-dependent CL.

We used 0.01 M luminol (Serva, Germany) in calcium- and magnium-free phosphate buffer, pH 7.0. Chemiluminescence was registered at 37°C in a Biolumat thermostated apparatus (model 9500, Bertold, Germany). The reaction was performed in the dark in red light.

Both spontaneous (SCL) and phagocytosis-dependent chemiluminescence (PCL) was determined. To assess PCL, we used a microbial suspension of *C.albicans* (1×10^8 particles/ml) inactivated by heating at 90°C for 60 min and opsonized with calf serum [7]. For launching the reaction, equal volumes (10 µl) of PEC, *C.albicans*, and luminol were mixed. In the case of SCL, 10 µl medium were added to the reaction mixture instead of *C.albicans*. Each sample was divided into two and the average value of CL activity was determined. The CL intensity was expressed as the number of pulses per min per 1×10^5 PEC.

RESULTS

As the first step, we investigated the nature of the changes in the cell composition of PEC and their CL activity in response to intraperitoneal injection of different materials during the first few hours. At least three samples of PEC were tested (individual or as a mixture obtained from 3-5 animals inoculated with different agents). Intact animals were used in the control experiments. Intraperitoneal injection of thioglycolate, casein, mineral oil, and BCG was shown to induce a substantial flow of N into the peritoneal cavity 3-5 hours after injection. The data on the cellular content of PEC of intact and inoculated animals are presented in Table 1. As follows from the table, the content of M and N in PEC of intact animals constitutes 65.3±4.5% and 5.6±2.9%, respectively. In inoculated animals, independently of the type of material injected, a drastic enrichment of the PEC population with N takes place, the neutrophil level rising to 81.2±8.3 - 96.3±2.8%.

Investigation of the CL activity of PEC from intact and inoculated animals enabled us to trace the following regularity: materials that stimulated a N flow, on the one hand, provoked a 4-10 fold increase in both SCL and PCL, on the other hand. This correlation may be accounted for by a higher CL activity of N compared to that of M of peritoneal exudate from intact animals [4].

In the next stage of the study we investigated the effect of different doses of PGE_2 on PEC from intact animals and animals inoculated with thioglycolate, casein, and BCG. As demonstrated, incubation of PEC from the animals inoculated with thioglycolate and BCG with PGE_2 results in a significant reduction of both the SCL and PCL of PEC. At the same time, we did not observe any inhibitory effect of PGE_2 on the CL activity of PEC from the animals inoculated with casein even for high doses of the agent: 10^{-5} - 10^{-7} M. It cannot be ruled out that the absence of any suppressive effect in the latter case may be related to a reduction (or complete disappearance) of the receptor function of casein-stimulated N [1]. As earlier reported, stimulated N exhibit the phenomenon of "stripping off" their receptors - in other words, they can destroy receptors by secreted enzymes or oxidants. It is possible that the prostaglandin receptors of casein-stimulated N are also subject to either hydrolysis or oxidation.

Examination of the effect of PGE_2 on PEC of intact animals did not reveal any decrease in their CL activity. This may be due to the fact that it is M which are mainly responsible for CL of PEC from intact animals. Since M secrete PGE themselves, they may be more resistant to its immunodepressive effect.

Thus, the study performed demonstrated a substantial enrichment of PEC with N and a marked enhancement of their CL activity in inoculated animals independently of the type of material used. In addition, we revealed a much higher sensitivity of N to a wide spectrum of PGE_2 doses in comparison with M. Finally, it was shown that N exhibit different degrees of sensitivity to PGE_2 when stimulated by different agents.

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Role of E-Type Prostaglandins in Tumor Cells During Contact with NK Cells *in Vitro*

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It has been demonstrated in our previous studies that highly malignant tumor cells, in contrast to cells of low malignancy, when in contact *in vitro* with NK cells as well as macrophages and neutrophils, rapidly begin (for several min) to secrete into the culture medium E-type prostaglandins (PGE), which suppress the cytotoxic activity (CTA) of NK cells [1,2]. We observed the active PGE release by highly malignant cells of Syrian hamster sarcoma, selected *in vivo* or transformed by the Rous sarcoma virus after their contact with NK cells. Information is available on the greater resistance to the cytotoxic effect of macrophages and neutrophils as well as NK cells exhibited by malignant tumor cells selected *in vivo* as compared to parental variants [4,8,9]. It has been shown in other reports [7,10] as well as in our own [2] that the resistance of several types of tumor cells is conditioned by their ability to suppress NK cells activity due to PGE secretion. It is obvious that PGE secretion induced by effector cells of the system of natural resistance is one of the protective mechanisms of tumor cells [1,6,9].

It is of interest to us to study the following aspects of the mechanisms of the interaction between

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