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MODULATION OF ROS/NO PRODUCTION BY MURINE PERITONEAL MACROPHAGES IN RESPONSE TO BACTERIAL CPG DNA STIMULATION

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Aim: **To investigate the features of metabolic activation induced by bacterial CpG DNA (bCpG DNA) in peritoneal macrophages (PMφs).** ***Methods:*** **Electron paramagnetic resonance spin-trapping technique using respective spin traps was applied to study the generation rate of reactive oxygen species and NO production by PMφs of BALB/c mice.** ***Results:*** **For the first time the capability of bCpG DNA isolated from *Bacillus subtilis* GP1-807-03 culture medium to elevate activity of NADP·H oxidase and inducible NO synthase in PMφs of normal and tumor-bearing mice have been demonstrated. The main differences in superoxide anion generation rate and production of NO by PMφs of normal mice and mice with transplanted solid Ehrlich carcinoma were showed. The effects of bCpG DNA stimulation *in vitro* on ROS and NO production by PMφs depended on concentration and time exposure with bCpG DNA. Furthermore, response of PMφs from tumor-bearing mice on bCpG DNA stimulation was delayed as compared to PMφs of normal mice.** ***Conclusion:*** **The present findings suggest that bCpG DNA have modulatory effect on ROS/NO production by PMφs from normal and tumor-bearing animals.**

Key Words: ***Bacillus subtilis*****, CpG DNA, electron paramagnetic resonance, nitric oxide, peritoneal macrophages, ROS, superoxide anion.**

For many years mononuclear phagocyte system was known as dynamic network with functionally heterogeneous scavenger cells of the monocyte/macrophage lineage, which are extremely critical for the integrity of host defense. During last two decades, it has become apparent that macrophages (Mφs) express complex functions such as phagocytosis, antigen presentation, production of cytokines capable to modulate the responses of other immune system cells and themselves as well, and bactericidal/tumoricidal activities [1–4]. Activated Mφs can kill some tumor cells directly or elicit tumor-destructive reactions, also they are able to stimulate antitumor cytotoxic lymphocytes [4]. Macrophage-mediated killing of tumor cells can be realized through different processes that can involve release of soluble factors (i.e., tumor necrosis factor-α (TNF-α), lysosomal enzymes, reactive oxygen species (ROS) and nitric oxide (NO) etc.) [2, 3, 5], activation of antibody-dependent cell-mediated cytotoxicity and phagocytosis [6].

ROS and NO are key elements in antimicrobial and antitumor macrophage-mediated host defense, at the same time they play a complex role in many diseases and in metabolic regulation [6, 7]. Under normal conditions, the integrity of this complex process is maintained, but during tumor progression these mechanisms may become altered, resulting in the inability of Mφs to lyse tumor cells [8].

The activation of Mφs tumoricidal activity is complex process that can be induced with different bacterial cell substances, including LPS and cytokines, such as IFN-γ [9, 10]. Bacterial DNA and synthetic oligonucleotides containing unmethylated CpG motifs are now recognized as potent immunostimulants [11–13]. Many investigations showed that CpG DNA is detected by majority of innate immune system cells, in particular dendritic cells, Mφs, and also B cells [11, 14]. In murine Mφs/monocytes CpG DNA causes the direct activation of nuclear factor κB (NFκB) and initiation of cytokine expression including TNF-α [15]. Yi et al. [16] have shown that leukocytes respond to CpG DNA through novel pathway involving the pH-dependent generation of intracellular ROS, which is essential for the activation of NFκB and all the downstream events observed after CpG DNA stimulation. However, Mφs expression of the inducible NO synthase and production of NO in response to CpG DNA is not direct but requires IFN-γ priming [17].

Pleiotropic immunostimulatory activity of CpG DNA provides a unique opportunity to design new immunotherapeutic approaches for treatment of cancer [18]. CpG DNA can be successfully used alone to locally activate the innate immunity and trigger a tumor-specific response or be applied in combination with different vaccines and monoclonal antibodies [11, 13, 19].

The goal of the present work is to study influence of bacterial CpG DNA (bCpG DNA) *in vitro* and *in vivo* on superoxide anion (SOA) and NO production by peritoneal Mφs (PMφs) of normal and tumor-bearing mice.

MATERIALS AND METHODS

Animals and reagents. BALB/c mice (9–12 weeks) were obtained from vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine (Kyiv, Ukraine). Animals were housed in standard facilities, with water and food *ad libitum*. All animals were maintained under strict ethical conditions according to International recommendations.

Hydroxylamine-2,2,6,6-tetramethyl-4-oxypiperidine was obtained from Institute of Chemical Physics, RAS, Russia.

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Abbreviations used: bCpG DNA – bacterial CpG DNA; CpG – cytosine-phosphodiester-bond-guanine or cytosine-phosphorothioate-bond-guanine; EPR – electron paramagnetic resonance; Mφs – activated macrophages; NFκB – nuclear factor κB; NO – nitric oxide, PMφs – peritoneal macrophages; PMA – phorbol myristate acetate; ROS – reactive oxygen species; SOA – superoxide anion; TNF-α – tumor necrosis factor α.

Bacterial strains and tumor models. *Bacillus subtilis* strain GP1-807-03 stored as strain IMB B-7108 in Bacterial Strain Collection of D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine (Kyiv, Ukraine) was used. *Bacillus subtilis* GP1-807-03 was routinely isolated from soil samples, characterized and cultivated in beef-extract broth (pH 7.0) at 37 °C during 8 days with moderate shaking (120 rpm).

In the study Ehrlich carcinoma strain obtained from the National Bank of Cell Lines and Tumor Strains of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine (Kyiv, Ukraine) was used. For tumor transplantation, 2.5×10^5 Ehrlich carcinoma cells in a volume of 100 µl of Hanks` balanced salt solution (HBSS, Sigma, USA) were inoculated subcutaneously (s.c.) into right flank of BALB/c mice.

Preparation and analysis of bacterial DNA. Bacterial extracellular DNA was isolated from *B. subtilis* GP1-807-03 culture medium on 9th day of cultivation according to [20].

Polyacrylamide gel (PAAG) electrophoresis was used for identification of DNA fragments in the samples [21]. To detect the presence of unmethylated CG-dinucleotides, restriction analysis of DNA with endonuclease Hpa II (Sigma, USA) [22] was applied with the next PAAG electrophoresis followed by ethidium bromide staining [21]. Restriction endonuclease digestion of DNA was performed according to the instructions of the manufacturer.

Isolated DNA was dissolved in 0.15 M endotoxin free phosphate buffer saline (PBS) at concentration 1 mg/ml. Samples of prepared DNA were stored at –20 °C. Repeated freezing of DNA samples was avoided.

Mice treatment. Two main groups of BALB/c mice (with s.c. transplanted Ehrlich carcinoma or without tumor) were used in the study. Normal mice (without tumor) received single s.c. injection of bCpG DNA at dose of 0.25 and 2.5 mg/kg body weight. Tumor-bearing mice were similarly injected with CpG DNA into site of tumor cell inoculation (on the 2nd day after tumor transplantation) or directly close to the tumor (on the 8th day after tumor transplantation). Control normal or/and tumor-bearing mice received injections with PBS.

On the 10th day after experiment beginning, all mice were sacrificed and peritoneal lavage fluids were collected. Tumors of all mice were stripped out and weighed accurately with torsion balance.

Preparation and stimulation of PMφs. Peritoneal lavage fluid cells were obtained from peritoneal cavity of immunized by CpG DNA or non-immunized normal and tumor-bearing mice. Purified PMφs were obtained from pooled peritoneal lavage fluid cells according to [23]. All cell samples were counted, adjusted in RPMI 1640 (Sigma, USA), supplemented with 2% FBS (Gibco, USA) to 1×10^6 cells/ml, and preincubated for 2 h. Before and after each experiment cellular viability was routinely measured by the trypan blue exclusion test. In all cases the viability was higher than 95%. For *in vitro* experiments, bCpG DNA (2 and 20 µg/ml) was added to respective PMφ samples and incubated for 2, 10, 20 and 30 min at 37 °C in a humidified atmosphere of 5% CO_2 with moderate shaking (100 rpm).

Electron paramagnetic resonance (EPR) spectroscopy. Electron paramagnetic resonance (EPR) spin-trapping technique was used to study SOA and NO production by PMφs [24, 25]. EPR spectra of liquid samples were recorded using an EPR spectrometer (RE 1307, Russia).

SOA generation analysis. Spin trapping measurements of SOA produced by PMφs were performed using hydroxylamine-2,2,6,6-tetramethyl-4-oxypiperidine. For recording at 20 °C, the sample was placed into a special custom-made quartz cavitiy (volume 170 µl) [24]. Briefly, 100 µl aliquots of PMφs suspension were mixed with 100 µl of reaction buffer (2×10^{-3} mol/l hydroxylamine, 2×10^{-3} mol/l NADP·H in phosphate buffer, pH 7.4 with 5×10^{-4} mol/l diethyltryaminepentaacetic acid). Estimations of SOA production were performed three times with a 1 min interval. The obtained data were expressed as SOA generation rate and measured as nmoles of produced SOA per 8.5×10^4 PMφs per min.

NO assay. The presence of NO in PMφs suspension was determined using diethyldithiocarbamate (DEDTC; Sigma, USA). Briefly, aliquots of 500 µl of PMφs suspension were mixed with 100 µl of reaction buffer (PBS, pH 7.4 with Fe^{2+} ions), placed into special metal molding tools and have been frozen in liquid nitrogen. Registration of NO was performed in Quartz Dewar (Brucker) cuvette at — 196 °C. The obtained data were expressed as nmoles of produced NO per 5×10^5 PMφs.

Statistical analysis. Data are expressed as means ± standard error of mean. For *in vivo* experiments, each group consisted of six mice. Each experiment was repeated at least twice. Dunnett multiple comparisons test and Student-Newman-Keuls test were used to comparisons of obtained data. Values $p < 0.05$ were considered as significant. Statistical analysis was performed using GraphPad InStat.

RESULTS AND DISCUSSION

Peculiarities of in vitro metabolic activation of PMφs by bCpG DNA. To evaluate the metabolic activity of murine PMφs, EPR spectroscopy was used to directly measure SOA and NO with respective spin traps. For this purpose, suspensions of PMφs obtained from peritoneal cavities of normal and tumor-bearing mice were stimulated in different expositions with 2 or 10 µg/ml CpG DNA from culture medium of *B. subtilis*. As shown in Fig. 1, incubation of PMφs with bCpG DNA was immediately reflected on their capability to generate SOA. However, this effect depended on bCpG DNA concentration and exposure time.

PMφs of normal mice responded to bCpG DNA stimulation as early as after 2 min of incubation (see Fig. 1, *a*). When 2 µg/ml bCpG DNA was added, the SOA generation rate of stimulated PMφs rapidly increased by 2.5-fold ($p < 0.01$) and dramatically decreased (almost 3.5-fold), when concentration of bCpG DNA was 10 µg/ml (absolute values were 1.25 and 0.15 nM/min, respectively, *versus* 0.5 nM/min

in control). After 10 min incubation of PMφs with bCpG DNA, opposite effect on SOA generation was observed. Drastic drop in SOA generation rate (to the level of 0.25 nM/min) was registered in PMφs stimulated by 2 μg/ml bCpG DNA, whereas 10 μg/ml bCpG DNA led to extremely rapid increase of SOA generation rate to 2.15 nM/min (higher than 14-fold as compared with previous value, $p < 0.01$). Subsequent responses of PMφs on stimulation with 2 μg/ml bCpG DNA during 20 and 30 min incubation were similar to previous observations (see Fig. 1, *a*). High bCpG DNA concentration (10 μg/ml) caused rapid decrease of SOA generation rate to control level (after 20 min incubation), and after 30 min incubation the values were lower than in control and averaged 0.3 nM/min. Thus, after 30 min incubation with bCpG DNA PMφs of normal mice in fact have use all resources for generation and release of SOA.

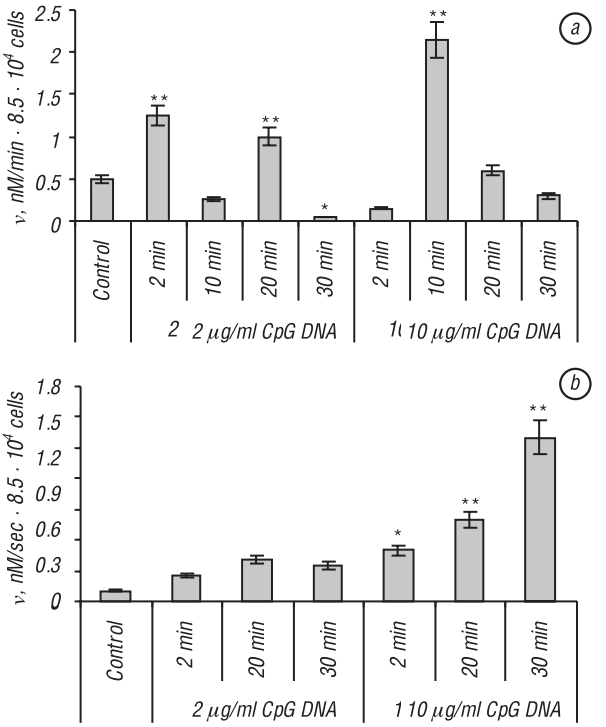

Fig. 1. Effect *in vitro* of bacterial CpG DNA on generation of SOA by PMφs of normal (*a*) and tumor-bearing (*b*) mice
*$p < 0.05$, **$p < 0.01$ compared to control

PMφs of tumor-bearing mice responded on bCpG DNA stimulation at different rate than PMφs from normal mice (see Fig. 1, *b*). It is important to note that unstimulated PMφs from mice with tumors showed very weak capability to generate SOA. The SOA generation rate of PMφs from control tumor-bearing mice was 0.1 nM/min — 5-fold lower than that in control normal mice PMφs (see Fig. 1, *a*, *b*). Stimulation of tumor-bearing mice PMφs with 2 μg/ml bCpG DNA weakly activated PMφs with insignificant differences in temporal characteristics (Fig. 1, *b*). When PMφs of tumor-bearing mice were stimulated by 10 μg/ml bCpG DNA, fluent increase of SOA generation rate with peak (1.6 *versus* 0.1 nM/s in control) after 30 min incubation was observed.

Thus, the highest values of SOA generation rate by PMφs were achieved with application of 10 μg/ml bCpG DNA after 10 min stimulation for PMφs of normal mice (4.3-fold higher comparing with control) and after 30 min stimulation (16-fold higher than in control) for PMφs of tumor-bearing mice.

Levels of NO production by murine PMφs are showed on Fig. 2. The same pattern was observed with NO production and SOA generation by normal mice PMφs *in vitro* stimulated with bCpG DNA (see Fig. 1, *a*, Fig. 2, *a*). Incubation of PMφs with 2 μg/ml of bCpG DNA caused a minimal activation but with marked inhibition in NO production (Fig. 2, *a*). After a 10 min exposure to 10 μg/ml bCpG DNA, PMφs developed the highest activity (2.7 nM/5 × 10^5 cells, $p < 0.01$) and subsequent gradual decrease in NO production was observed. So, dramatic decrease of NO production by PMφs stimulated during 30 min with 10 μg/ml bCpG DNA was shown, evidencing on rapid depletion of PMφ ability to generate and release NO. Along with that, NO production by PMφs stimulated with 2 μg/ml bCpG DNA had oscillatory character with gradual increase of NO levels (see Fig. 2, *a*).

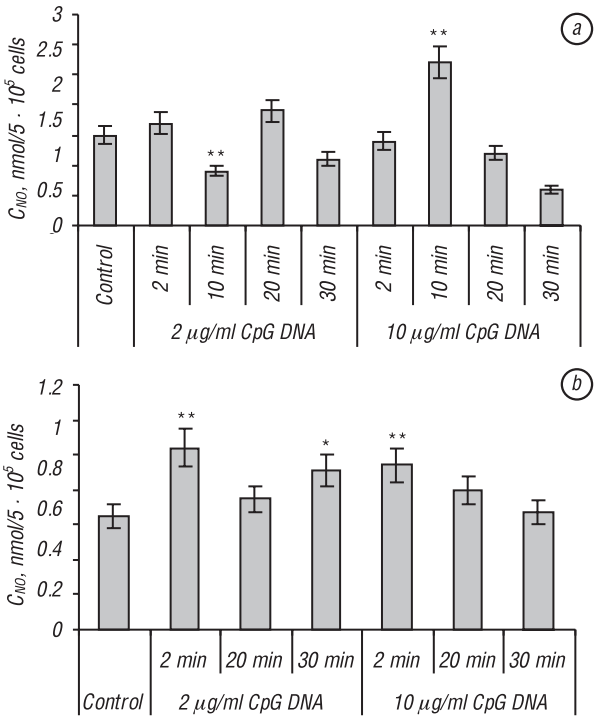

Fig. 2. Effect *in vitro* of bacterial CpG DNA on NO production by PMφs of normal (*a*) and tumor-bearing (*b*) mice
*$p < 0.05$, **$p < 0.01$ compared to control

Fig. 2, *b* compares the time course stimulation of PMφs of tumor-bearing mice. Bacterial bCpG DNA at concentration of 2 μg/ml stimulated *in vitro* NO production by PMφs insufficiently during all the time of incubation. Nevertheless, already after 2 min incubation NO levels were significantly increased (1.6-fold, $p < 0.01$) compared to control. At first, treatment of PMφs by higher concentration of bCpG DNA (10 μg/ml) weakly but significantly stimulated capability to produce of NO (0.94 nmol/5 × 10^5 cells after 2 min incubation, $p < 0.01$) and after 30 min incubation NO production values were close to control ones.

Thus, effects of bCpG DNA stimulation *in vitro* on metabolic activity of PMφs depended on concentration and period of exposure to bCpG DNA. Higher concen-

tration of bCpG DNA caused strong metabolic activation of PMφs of normal mice as early as after 10 min stimulation and resulted in subsequent anergy of PMφs displayed in dramatic inability to generate and release SOA and NO. Tumor growth was also notably reflected in bCpG DNA-induced metabolic activation of PMφs. Response of tumor-bearing mice PMφs on bCpG DNA treatment was slow and characterized by gradual enhancement or extinction of metabolic activity.

Effects of bCpG DNA administration in vivo on metabolic activity of murine PMφs. To evaluate possible effects of *in vivo* bCpG DNA application on metabolic activity of murine PMφs, normal and tumor-bearing mice were s.c. immunized with single injection of bCpG DNA at the doses of 0.25 and 2.5 mg/kg of body weight. To assess capability of bCpG DNA to activate PMφs in mice with different stages of tumor growth, immunization was performed at 2nd and 8th days after tumor cell inoculation. Metabolic activity of PMφs of normal mice was analyzed at 2nd and 8th days after immunization in order to evaluate duration of bCpG DNA stimulatory effects.

Total number of peritoneal lavage fluid cells obtained from peritoneal cavities of normal or tumor-bearing mice at different times after s.c. injection of a single dose of bCpG DNA was not significantly changed as compared to respective controls, except for statistically significant increase of total cellularity ($p < 0.05$) at 8th day of observation in normal mice injected by bCpG DNA at a dose of 0.25 mg/kg of body weight (Table). Our previous studies also did not show any significant changes in cell content of peritoneal lavage fluid after s.c. injection with bCpG DNA at a dose of 5 mg/kg body weight (approximately 100 μg/mouse), whereas intraperitoneally injected mice developed strong inflammatory response determined as a transitory aseptic peritonitis [27].

Table. Effect of bacterial CpG DNA administration on cellularity of peritoneal lavage fluid of immunized mice

Group of mice	Day of CpG DNA injection	Dose of CpG DNA, mg/kg	Total number of peritoneal lavage fluid cells ($\times 10^6$/mice)
mice without tumors	2nd	0.25	13.1 ± 3.4
		2.5	12.3 ± 3.2
	8th	0.25	14.1 ± 1.7*
		2.5	10.3 ± 1.7
	control	–	9.6 ± 1.3
mice with tumors	2nd	0.25	12.7 ± 5.3
		2.5	7.9 ± 3.6
	8th	0.25	9.5 ± 2.8
		2.5	10.8 ± 0.5
	control	–	9.2 ± 1.0

*$p < 0.05$ compared to respective control

As shown in Fig. 3, a, in response to stimulation with bCpG DNA, PMφs of normal mice displayed no significantly increased capacity to generate and release of SOA as compared with control. Only at the 8th day after challenge with bCpG DNA (0.25 mg/kg of body weight) the modest and statistically significant elevation of SOA production was observed (0.8 *versus* 0.45 nM/min in control, $p < 0.05$). These observations indicate that *in vivo* application of relatively low doses of bCpG DNA (0.25–2.5 mg/kg body weight) results in actually insignificant capability of PMφs to generate and produce ROS including SOA at least during 8 days after immunization.

PMφs of control tumor-bearing mice in fact showed very weak activity in SOA production (Fig. 3, *b*). In contrast, PMφs obtained from tumor-bearing mice injected by bCpG DNA at the 2nd day after tumor cell inoculation were able to generate SOA and demonstrated most evident increase in SOA generation rate (0.3–0.5 compared to 0.15 nmol/min in control). If tumor-bearing mice were injected with bCpG DNA at the 8th day after tumor transplantation, no increase in ability of SOA generation by PMφs was observed.

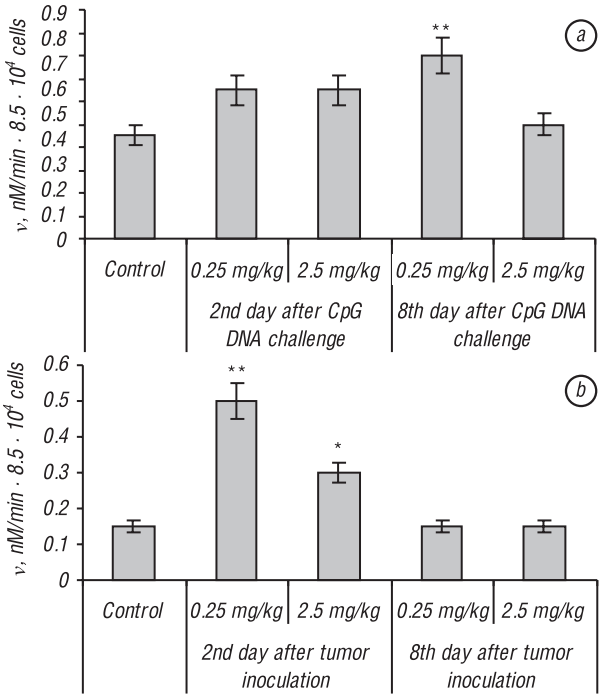

Fig. 3. Effect of bacterial CpG DNA administration on SOA generation rate by PMφs of normal (*a*) and tumor-bearing (*b*) mice
*$p < 0.05$, **$p < 0.01$ compared to control

Application of bCpG DNA in normal mice had a marked dose-dependent effect on NO production by PMφs (Fig. 4, *a*). Administration of bCpG DNA at a dose of 0.25 mg/kg body weight resulted in significant increase of NO production that averaged 3.7 ($p < 0.01$) and 1.94 nM at the 2nd and 8th days after bCpG DNA challenge, respectively, whereas in control group — only 1.45 nM. As shown in Fig. 4, *a*, injection of bCpG DNA (2.5 mg/kg body weight caused) resulted in marked inhibition of NO production by PMφs (0.82 and 1.1 nM *versus* 1.45 nM in control) at both days of observation.

PMφs from tumor-bearing mice after challenge with bCpG DNA at a dose of 2.5 mg/kg body weight responded by increased NO levels as compared to control (Fig. 4, *b*). Administration of bCpG DNA (0.25 mg/kg body weight) resulted in 2.5-fold increase of NO levels at the 2nd day after tumor transplantation and 4-fold increase of NO production at the 8th day after tumor transplantation ($p < 0.01$). When mice were injected with 2.5 mg/kg bCpG DNA, only 2-fold increase of NO levels was observed at the 2nd day after tumor transplantation.

Thus, application of bCpG DNA *in vivo* resulted in significant changes of PMφs metabolic activity. PMφs of normal mice injected with bCpG DNA didn't show marked increase of SOA generation rate, except that at the 8th day

after challenge with 0.25 mg/kg bCpG DNA, whereas significantly increased SOA-producing activity of PMφs of tumor-bearing mice was observed only when bCpG DNA immunization was performed at the 2nd day after tumor transplantation. Augmentation of NO production by PMφs was observed in normal mice at the 2nd day and in tumor-bearing mice - on both terms of observations after immunization with 0.25 mg/kg bCpG DNA.

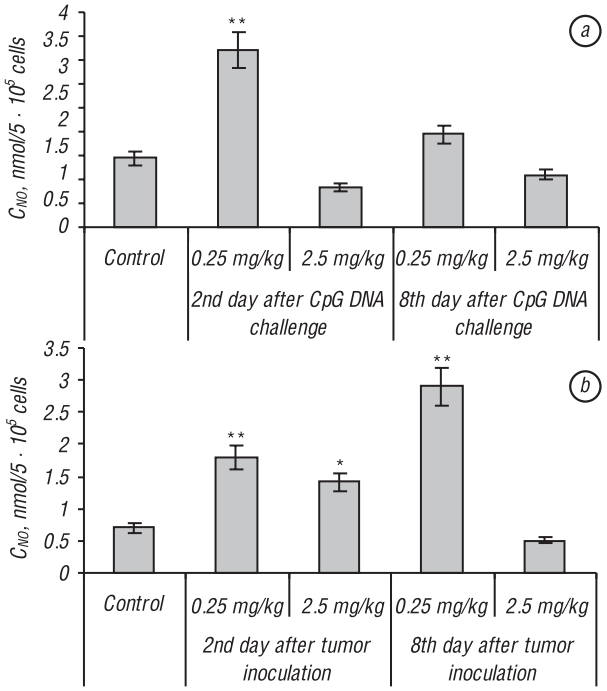

Fig. 4. Effect of bacterial CpG DNA administration on NO production by PMφs of normal (*a*) tumor-bearing (*b*) mice
*$p < 0.05$, **$p < 0.01$ compared to control

Next, we evaluated whether single dose injection of bCpG DNA has infuence on tumor growth in mice with solid Ehrlich carcinoma. The results presented in Fig. 5 demonstrated that mice injected with 0.25 mg/kg bCpG DNA had smaller tumors compared to control animals. If mice were challenged with 10-fold higher dose bCpG DNA, the trend to stimulation of tumor progression was observed (see Fig. 5). These data show that treatment of mice by higher doses of bCpG DNA (2.5 mg/kg in this investigation) possibly stimulate tumor growth probably because of decreased functional activity of cells of immune system (in particular, PMφs).

Mφs have the opportunity to produce SOA and NO in nearly equimolar amounts and thus can be generators of the particularly destructive products. Moreover, Adachi et al. [28] showed that the highest degrees of tumor-associated Mφs phagocytosis are associated with favorable prognosis. In this regard, Fiumara et al. [29] demonstrated that distant metastases developed in none of the cancer patients with *in situ* evidence of phagocytosis of neoplastic cells by tumor-associated Mφs. Baskić et al. [30] have showed that functional properties of Mφs isolated from different anatomical sites of cancer patients possess different functional properties. It has been found that tumor-associated Mφs display a diminished capacity to produce NO. Along with this, Brignole et al. [31] have presented evidence that depletion of Mφs led to a complete loss of antitumor efficacy in neuroblastoma-bearing mice injected with liposome-CpG-myb-as. Activation of Mφs is an attractive approach in the development of cancer immunotherapy [32–34]. Indeed, evidence from many studies showed that activated Mφs are capable to lyse selectively tumor cells *in vitro* [33–36] and play an important role in the immune-mediated destruction of tumor *in vivo* [37–40]. In clinical studies Mφs are found to play a role in the mechanism of the antibody-mediated tumor killing [41, 42]. Taken together, these studies suggest that activation of Mφs could be fine strategy for the development of cancer immunotherapy.

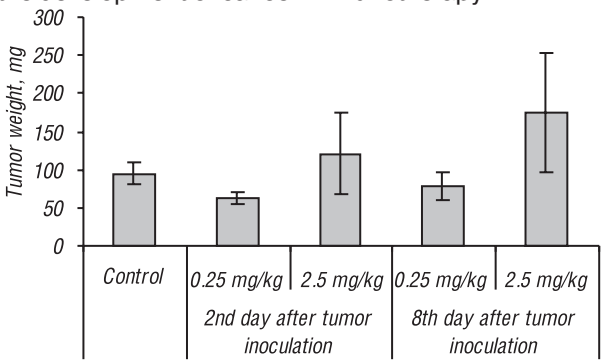

Fig. 5. Effect of single dose bacterial CpG DNA administration on Ehrlich carcinoma growth *in vivo*

There is strong evidence that during tumor progression, complex process leading to ROS and NO production may become altered, resulting in the inability of the Mφs to kill tumor cells. It is well-known that factors produced by tumors (transforming growth factor-β, prostaglandin-E_2, interleukin-10, and phosphatidyl serine) may lead to dysregulaton of complex tumoricidal machinery of Mφs. Furthermore, neoplastic cells have the capacity to impair the production of T cell-derived cytokines (e. g., interferon-γ) important for activation of Mφs [30, 43, 44].

The results presented herein provide further insight into the immunomodulatory effects of bCpG DNA and perspectives of its application for cancer immunotherapy. For better understanding of immunomodulatory activities and immunotherapeutical potential of bCpG DNA, it is crucial to elucidate the immune system reaction on immunization with bCpG DNA with a focus on defense oriented molecules such as ROS and NO produced by Mφs. In the present study we showed that bCpG DNA can induce SOA and NO production by PMφs in a dose- and time-dependent way. Also, it is important to note, that tumor growth have a strong inhibitory effects on metabolic activity of both intact and bCpG DNA stimulated PMφs. These findings once again confirm the probability of inhibitory influence of developing tumor on host's immunity and suggest that immunostimulatory bCpG DNA may be useful for immunotherapeutic treatment of malignant tumors. Furthermore, Auf G. et al. [45] have showed that Mφs play a critical role in mediating the CpG DNA effects. In this study, activated by CpG DNA Mφs but not B or T cells caused the early phase of tumor rejection. Our previous investigations provide the evidence that *B. subtilis* culture medium CpG DNA possesses strong

immunostimulatory activity and can be applied as a potent immunoadjuvant [12, 27].

Also, it is important to emphasize that despite earlier hopes that the induction of nonspecific immunity might be a valuable tool in the fight against infectious diseases and tumors with the subsequent production of reactive oxygen and nitrogen intermediates, it is now clear that such responses can be extremely dangerous unless carefully controlled. Unfortunately, an uncontrolled production of ROS, such as SOA, cause vascular endothelial damage and strong inflammatory reactions, NO can profoundly effect on blood pressure and flow, inhibits platelet adhesion and aggregation that can be fatal for organism [5, 46].

In conclusion, bCpG DNA can induce significant metabolic activation of PMφs from normal and tumor-bearing mice. In this scenario, it is apparent that administration of immunostimulatory CpG DNA to a tumor-bearing immunocompetent host would result the metabolic activation of Mφs along with other immune system cells to destroy cancer cells. Thus, results obtained may be important for future therapeutic approaches that implicate immunostimulatory CpG DNA.

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МОДУЛЯЦИЯ ПРОДУКЦИИ РФК И ОКСИДА АЗОТА ПЕРИТОНЕАЛЬНЫМИ МАКРОФАГАМИ В ОТВЕТ НА ВОЗДЕЙСТВИЕ БАКТЕРИАЛЬНОЙ CPG ДНК

Цель: **изучить особенности метаболической активации перитонеальных макрофагов (ПМФ) в ответ на воздействие, вызванное CpG ДНК бактериального происхождения.** ***Методы:*** **для исследования скорости генерирования радикальных форм кислорода (РФК) и продукции оксида азота (NO) ПМФ мышей линии BALB/c был применен метод электронного парамагнитного резонанса с использованием спиновых ловушек.** ***Результаты:*** **впервые в эксперименте продемонстрирована способность бактериальной CpG ДНК, выделенной из культуральной жидкости *Bacillus subtilis* GP1-807-03, повышать активность НАДФ·Н-оксидазы и индуцибельной NO-синтазы ПМФ у мышей с солидной карциномой Эрлиха и интактных мышей. Отмечены различия в скорости генерирования супероксидных радикал-анионов и уровне продуцирования NO ПМФ у животных с карциномой Эрлиха по сравнению с контрольными животными, что выражалось в замедлении ответа на воздействие бактериальной CpG ДНК. Выявлена концентрационная и временная зависимость влияния бактериальной CpG ДНК *in vitro* на продукцию РФК и NO ПМФ.** ***Выводы:*** **полученные данные свидетельствуют о том, что бактериальная CpG ДНК обладает модулирующим влиянием на продукцию РФК и NO ПМФ *in vitro* и *in vivo*.**

Ключевые слова: ***Bacillus subtilis*****, CpG ДНК, электронный парамагнитный резонанс, оксид азота, перитонеальные макрофаги, РФК, супероксидный анион.**