

MAGNETICALLY SENSITIVE NANOCOMPLEX ENHANCES ANTITUMOR EFFICACY OF DENDRITIC CELL-BASED IMMUNOTHERAPY

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Background: One of the major factors restricting *in vivo* efficacy of dendritic cells (DCs) based immunotherapy is the inefficient migration of these cells to the lymphoid tissue, wherein DCs activate antigen-specific T cells. A fundamentally new approach for the possibility of enhancing the antitumor effects of DC-based immunotherapy may be the use of magnetically sensitive nanocomplexes to increase the target delivery of DCs to the lymph nodes of the recipient. **Aim:** To study the antitumor and immunomodulatory effects of the DC-nanovaccine with magnetosensitive properties and its influence on the immunosuppressive tumor microenvironment in mice with sarcoma 37. **Materials and Methods:** The antitumor, antimetastatic and immunomodulatory effects of DCs loaded with magnetic nanocomplex under magnetic field (MF) control in mice with sarcoma 37 have been investigated. **Results:** Combined therapy contributed to a significant reduction in tumor volume and weight compared to the control group of mice and mice that received the DC vaccine without MF. Therapy with magnetically sensitive DC nanovaccine with and without the addition of the MF was accompanied by a significant down-regulation of the level of FoxP3, transforming growth factor β , interleukin (IL)-10 and vascular endothelial growth factors, mRNA expression in tumor tissues. A significant increase in interferon- γ and IL-4 mRNA expression was found in mice treated with the magnetically sensitive DC nanovaccine under MF control. **Conclusion:** A significant increase in the antitumor efficacy of the DC vaccine can be achieved using magnetosensitive nanocarriers of tumor antigens under MF control.

Key Words: dendritic cells, iron oxide nanoparticles, mouse sarcoma, magnetic field, antitumor effect.

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Cell-based technologies represent a promising approach for cancer immunotherapy among special places that are occupied by the use of *ex vivo*-generated, tumor antigen-pulsed dendritic cells (DCs) [1, 2]. The exceptional properties of DCs allow us to consider them as the most specialized antigen-presenting cells, which play a central role in the modulation of the immune response and thus, can be wisely utilized as an immunotherapeutic strategy for cancer regimens [3]. However, despite recent progress that has been achieved in the DC vaccination field, clinically effective DC immunotherapy as a monotherapy for a majority of tumors remains a distant goal. One of the major factors that contribute to the restriction of the efficacy of DCs immunotherapy *in vivo* is the ineffective migration of DCs to the lymph node leading to non-sufficient activation of antigen-specific T cells. Thus, improving the DC vaccine delivery to the lymphoid tissue could significantly increase the effectiveness of immunotherapy. A widely used approach to enhance DCs migration to lymphatic tissue is pre-injection of pro-inflammatory cytokines such as tumor necrosis factor or enhancing the expression levels of specific DC homing receptors that can also facilitate migration. However, *in vivo* DCs migration remains unsatisfactory with a typical

rate of less than 4–5% when administered via intradermal injection [4].

A fundamentally new approach for the possibility of enhancing the antitumor effects of DC-based immunotherapy may be the use of magnetically sensitive nanocomplexes to increase the targeted delivery of DCs to the lymphoid tissue of the recipient. Such nanocomplexes consist of magnetic (iron oxide) nanoparticles (NP) and tumor antigens, which should be used for DCs loading. It should be noted that several works have demonstrated the increase in the targeted delivery of DCs to lymphoid tissue by using the metal NP under magnetic field (MF) control [4, 5].

Here, we developed the antitumor DC-based nanovaccine using magnetic NP in combination with magnetic pull force to enhance DCs migration to lymphoid tissue. We hypothesized that an increase in the delivery of the DCs to the recipient's lymphoid tissue could enhance the efficacy of DC therapy.

The purpose of this work is to study the antitumor and immunomodulatory effects of the DC-nanovaccine with magnetosensitive properties and its influence on the immunosuppressive tumor microenvironment (TME) in mice with transplanted Sa-37.

MATERIALS AND METHODS

In vivo animal studies. All experiments were performed according to the requirements of the local Ethic Commission of the National Cancer Institute of Ukraine and the principles of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes. The experimental studies were carried out on 60 CBA mice, males ($n = 30$) and females ($n = 30$) weighing 18–22 g, aged 1.5–2 months, bred

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Abbreviations used: DCs – dendritic cells; IL – interleukin; IFN – interferon; LPTC – lyophilized tumor cells; MIV – magnetic induction vector; MF – magnetic field; MNC – magnetic nanocomplex; NP – nanoparticles; TGF – transforming growth factor; TME – tumor microenvironment; VEGF – vascular endothelial growth factors.

in the vivarium of the National Cancer Institute of Ukraine. Mice were inoculated with $9 \cdot 10^5$ sarcoma 37 (Sa-37) cells into the thigh of the left paw. The animals were divided into four groups of ten mice each: 1st — control (no treatment); 2nd — mice treated with DCs loaded with lyophilized tumor cells (LPTC); 3rd — mice treated with DCs loaded with magnetic nanocomplex (DCs + MNC); 4th — mice treated with DCs loaded with MNC and exposed to the MF. On day 7 after tumor inoculation, DCs were administered intradermally 3 times every 3 days. The scheme of the experiment is shown in Fig. 1.

The animals were sacrificed on day 28 after tumor cells inoculation and the tumors were removed for weight measurement. The tumor volume was determined during its growth stages. The tumor size was measured by the digital caliper and the tumor volume was calculated according to the formula 1:

$$V_i = \frac{\pi}{6} D_i^3, (1)$$

V_i — a volume of the tumor; D_i — diameter of the tumor at the certain time t_i , cm

At the same time, inguinal lymph node tissue was obtained from mice to determine the level of cytokines mRNA expression.

Obtaining of DCs. DCs isolation was performed according to the previously described method with minor modifications [6]. Single-cell suspensions were made from spleens of intact CBA mice by mechanical disruption over nylon mesh. Cell suspensions of spleens were cultured at $5 \cdot 10^6$ cells per ml of complete culture medium (RPMI 1640 medium, with 100 units of penicillin and 100 µg of streptomycin per ml, 10 µM 2-mercaptoethanol, and 10% fetal calf serum; Sigma, USA) for 24 h in Petri dishes. After incubation, cells were centrifuged at 600 g for 20 min in gradient density of 14.5% metrizamide (Sigma, USA). The interphase cells were morphologically identified as DCs (> 85%).

In vitro DCs studies. To evaluate the ability of DCs to engulf iron oxide $\gamma\text{-Fe}_2\text{O}_3$ maghemite (Sigma-Aldrich, USA) NP (< 50 nm diameter), the following studies were carried out. DCs were incubated with NP in amounts of 8, 12 or $24 \cdot 10^{-12}$ g per cell during 24 h. To investigate the presence of NP in DCs, the cells were fixed and stained with the detection method of Prussian blue iron stain and a carmine-containing dye [7]. NP loaded DCs were fixed with 4% paraformaldehyde for 30 min at room temperature, washed, and incubated with 2% potassium ferrocyanide in 2% hydrochloric acid for 30 min, and washed again. Primo Star direct microscope (Carl Zeiss, Germany) and AxioVision software were used to analyze the cytological preparations at $\times 400$ magnification.

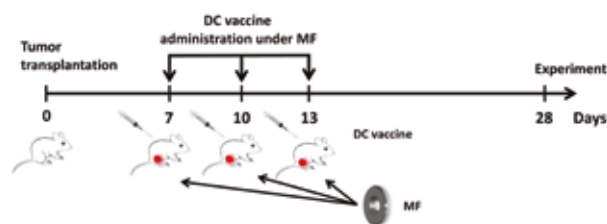


Fig. 1. Scheme of the experiment

Cell viability was determined after staining with the vital dye propidium iodide (PI) using a flow cytometer FACSCalibur (BD, USA).

To study the possibility of DCs loaded with NP to change the spatial distribution with a change of the magnetic induction vector (MIV), a disc neodymium magnet with a tetragonal $\text{Nd}_2\text{Fe}_{14}\text{B}$ crystal structure and induction 84 mT was utilized. For this, the magnet was leaned against a Petri dish with DCs from different sides. The DCs distribution was determined using an Axiovert 40 C inverted microscope (Carl Zeiss, Germany) at $\times 100$ magnification.

LPTC and MNC for DCs loading. The tumor nodes in CBA mice were removed on day 14 after inoculation of Sa37. Suspension of tumor cells was prepared using mechanical disintegration of tissue followed by filtering through nylon filter with a diameter of pores 1 mm. The obtained suspension was washed in Ringer's solution twice by centrifugation at 2500 g. Tumor cells were frozen in Ringer's solution at -20°C in Petri dishes (thickness of the layer — 5–8 mm). Frozen tumor cells were subjected to vacuum sublimation drying for 24 h. Obtained LPTC were used to load DCs or for MNC preparation.

MNC consisted of NP and LPTC of Sa-37. For MNC preparation, LPTC and NP were subjected to mechanical treatment in the microvibromill Lotus (Lotus, Ukraine) [8]. For this purpose, 10 mg of LPTC and 1.6 mg of NP were put into a chamber with grinding pellets (Retsch, Germany). The energy supplied to the chamber content was 20 W/g at the frequency of 35 Hz while the vibration amplitude was 10 mm. The process lasted 5 min. After treatment, 5.6 mg of MNC was diluted with 5 ml Ringer's solution. Then, DCs were incubated at 37°C for 24 h in RPMI-1640 medium containing MNC at a final concentration of NP of $8 \cdot 10^{-6}$ g per 10^6 of cells and $5 \cdot 10^{-5}$ g of LPTC per 10^6 of cells. Then DCs were washed, suspended in Ringer's solution, and administrated intradermally.

MF. In some animals, the inguinal lymph nodes, which are regional to the tumor, were exposed to the constant MF (magnetic force 20 pN at average) for 1 h at a temperature not exceeding 37°C .

mRNA expression of cytokines and FoxP3. The levels of mRNA expression of transforming growth factor- β (TGF- β), interleukin (IL)-10, transcription factor FoxP3, vascular endothelial growth factor (VEGF)- α in the tumor tissue and IL-4, interferon (IFN)- γ in the lymph nodes of mice were determined by the real-time PCR method using 7300/7500 Real-Time PCR Systems (Applied Biosystems, USA) and specific primers and fluorochrome SYBR Green. The primer sequences were selected using Primer Express[®] Software v3.0 and synthesized by Applied Biosystems (USA). The accounting of the obtained results was carried out according to the recommendations of the equipment manufacturer. The level of cytokine gene expression was assessed using the $\Delta\Delta\text{Ct}$ method with normalization for the expression of the control GAPDH gene.

Statistics. Statistical analysis was performed using Statistica version 10 (StatSoft Inc, USA) and MedCalc 12.1 (MedCalc Software Ltd, USA). Gaussian distribution of the group was checked with Shapiro–Wilk test. Statistical analysis included Mean $\pm$ SE for Gaussian distribution

and Median $\pm$ Percentiles (Q_1 and Q_3) for non-parametric data. To compare the data in three groups, we used One-way ANOVA with Tukey post-hoc test for Gaussian distribution and Kruskal–Wallis test for nonparametric ones. Null-hypothesis of variables equality was rejected when $p < 0.05$.

RESULTS

DCs studies. It is known that DCs engulf nanosized particles by endocytosis and pinocytosis. As a result of incubation of mouse DCs with NP for 24 h, samples of DCs cultures with incorporated NP were obtained. Prussian blue staining showed that DCs were capable of high levels of NP uptake. DCs in the cytological preparations were colored in red, and NP — in deep blue (Fig. 2, *a*). The optimal number of iron oxide NP for the DCs loading and co-culture time has been determined. After 24 h of DCs incubation with $8 \cdot 10^{-12}$ NP per cell, nearly all cells were shown to contain them. Incubation of DCs with this number of NP did not affect their viability. At the same time, larger amounts of NP (12 and $24 \cdot 10^{-12}$) caused the aggregation and death of up to 30% of the DCs. In addition, no changes of morphological characteristics were detected in DCs after their incubation with MNC for 24 h. In further studies, we used NP for the MNC preparation for DCs loading purposes at the rate of $8 \cdot 10^{-12}$ NP per cell.

The next step was to detect the possibility of DCs loaded with NP to change the spatial distribution with a change of the MIV. The results of *in vitro* test are presented in Fig. 2. The images in Fig. 2, *b* clearly show the location changes of the NP to the DCs towards the direction of the MF at each point of space. The pilot analysis showed that the spatial distribution of DCs had been consistently changing with a change of the MIV.

Our analysis also showed that the DC viability did not alter either due to the incubation with MNC and/or influence of constant MF of the magnet.

The ability of the DCs loaded with NP to follow the MIV opens the possibility of improving their targeted delivery to the lymph nodes using MF exposure. As seen in Fig. 2, *b*, NP follow the direction of the changed MIV that could result in an improvement of their targeted delivery to the lymph nodes. Thus, we assumed that the efficacy of the antitumor nanovaccine based on DCs and MNC could be controlled by the constant MF. Based on *in vitro* tests for the magnetosensitive nanovaccine, the following *in vivo* experiments have been provided.

Antitumor effect of DCs loaded with MNC under MF control. We used a magnetosensitive nanovaccine consisting of DCs loaded with MNC for *in vivo* studies in CBA mice with transplanted Sa-37. The inguinal lymph nodes of the fourth group of mice that are regional to the tumor were exposed to the MF of neodymium magnet for 1 h. The tumor volume was measured in the dynamics of its growth. At the end of the experiment, the tumors were weighed.

The results of our studies showed that intradermal administration of DCs loaded with MNC followed by exposure to MF contributed to a significant reduction in tumor volume compared with the control group of mice (Fig. 3, *a*).

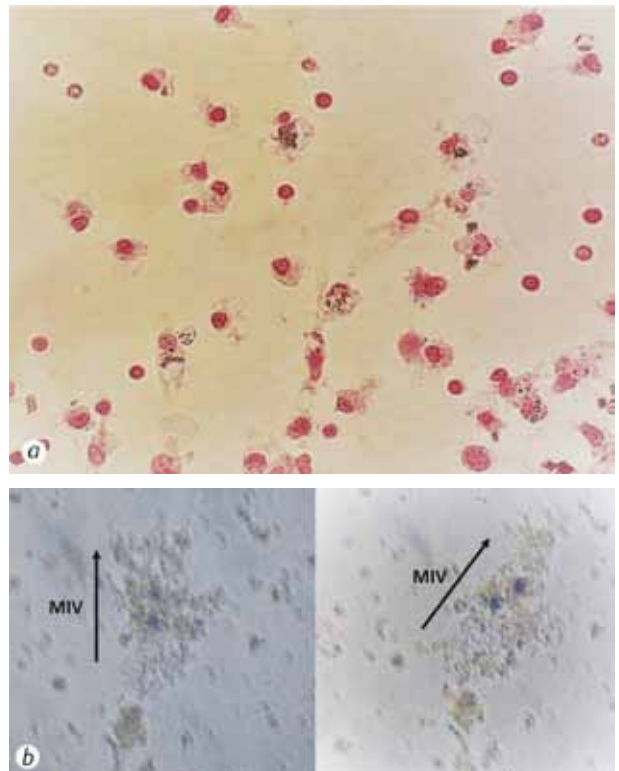


Fig. 2. *a*: Cytology of the mouse splenic DCs labeled with NP after 24 h incubation. Prussian blue staining to visualize the captured NP (blue), $\times 100$; *b*: Change in the spatial distribution of DCs labeled with iron oxide NP along with changes of the MIV under inverted microscope, $\times 100$

Starting from the 17th day, statistically significant difference in tumor volume was observed between the group of mice treated with the DC nanovaccine under MF control and all other groups of mice, $p < 0.05$. It should be noted that tumor volume in animals of this group remained the smallest during the entire observation period. Moreover, mice treated with the DC nanovaccine loaded with MNC under MF control, tumor weight was the lowest compared to other groups of mice (Fig. 3, *b*).

Immunosuppressive factors in the tumor micro-environment. TME forms one of the main obstacles in effective cancer immunotherapy [9]. We have analyzed the expression of mRNA of cytokines with immunosuppressive properties, VEGF- α and nuclear factor FoxP3 in the TME without dividing the immunosuppressive response into tumor cell-, stromal cell- or immune-cell associated.

In mice, nuclear factor FoxP3 is expressed only by Treg cells with $CD4^+ TCR\alpha\beta^+$ phenotype, which have suppressor activity [10]. In our studies, a significant decrease in the FoxP3 mRNA level in the tumor occurred in mice receiving monotherapy with DCs loaded with LPTC and MNC (Fig. 4). In mice treated with DCs loaded with MNC and exposed to MF, the decrease in FoxP3 mRNA level was in line with the trend. In mice treated with DC/MNC or DC/MNC under the action of the MF, the decrease in FoxP3 mRNA level was insignificant. The decrease in FoxP3 mRNA expression in the tumor indicates a decrease in the negative effect of Treg cells on the local antitumor immune response.

The immunosuppressive cytokines, such as IL-10 and TGF- β , are secreted by tumor cells and Treg

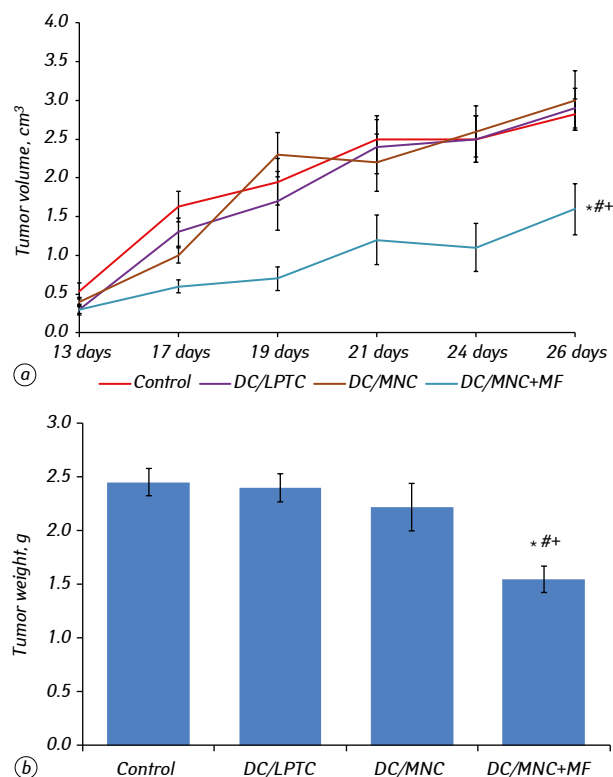


Fig. 3. Evaluation of *in vivo* antitumor efficiency of DCs loaded with MNC in mice with Sa-37: *a* — The dynamics of Sa-37 growth; *b* — weight of the dissected tumors. Data are presented as the Mean $\pm$ SE. * $p < 0.05$ in comparison with control group of mice; [#] $p < 0.05$ in comparison with DC/LPTC group of mice; ⁺ $p < 0.05$ in comparison with DC/MNC group of mice)

and inhibit the maturation of DCs, T cell and NK cell function in TME [11]. In our study, DC-based monotherapy, as well as combined therapy, contributed to a significant decrease in the TGF- β mRNA level in TME. It was also found that any therapy regimen contributed to a significant decrease in the level of IL-10 mRNA expression. However, it should be noted that the downregulation of the mRNA expression of the immunosuppressive trio in TME — FoxP3, TGF- β and IL-10, didn't play an essential role in the realization of the antitumor effect of combined immunotherapy based on DCs and MF.

DC-based therapy also influenced the expression level of VEGF, which is the main factor in the growth of the vessel in the tumor and supports the nutritional supply of tumor cells. The decrease in the level of VEGF mRNA expression in tumors of mice treated with DCs and magnet was insignificant.

Expression of cytokines mRNA in the lymph nodes. It is known that DCs are endowed with a unique potency to prime systemic antitumor T cell immunity, as well as to orchestrate T cell expansion, their functional polarization, and effector activity in draining lymph nodes. The interaction between DCs and T-lymphocytes occurs in the T-cell zones of the lymph nodes under the modulating effect of cytokines [12]. The level of IL-4 and IFN- γ production by peripheral T-lymphocytes after contact with DCs largely determines the activation of certain T-lymphocyte subpopulations.

As shown by our results, the magnetic-enhanced effect of DC-nanovaccine is accompanied by an increase in mRNA levels of both the pro-inflammatory cytokine IFN- γ and the anti-inflammatory cytokine IL-4 in the inguinal lymph nodes of tumor-bearing mice (Fig. 4, *b*). It should be noted that such activation of cytokine genes was observed only in animals that received DC-nanovaccine followed by exposure to the MF. In mice treated with DC-nanovaccine, no changes in the expression of IFN- γ and IL-4 mRNA levels in comparison with the control group of animals were detected.

DISCUSSION

DCs are central regulators of the adaptive immune response, and as such are necessary for T cell-mediated cancer immunity. DCs are thought to endocytose dead neoplastic cells or cellular debris and transport cancer-associated antigens to the draining lymph node where T cell priming and activation can occur. To ensure effective DC-based immunotherapy, sufficient amounts of inoculated DCs should migrate to lymph nodes to activate T cells. The numbers of DCs that ultimately end up in the T-cell zone have been shown to determine the magnitude of T-cell proliferation and effector response [13]. High DCs number increases the probability of DCs–T-cell encounters delivering a sustained stimulation through successive interactions and reducing a competition among T cells. Conversely, low numbers of poorly stimulatory DCs induce abortive T-cell proliferation and tolerance [14]. However, in tumor-bearing animals, the migratory activity of DCs is significantly reduced. In our previous studies, we have revealed that the migratory activity of DCs loaded with tumor cell lysate in animals with transplanted Sa-37 is significantly impaired in comparison with intact animals [15]. Therefore, the search for the ways to enhance the delivery of DCs to lymphoid tissue to drive antitumor responses remains an essential step for such vaccines efficacy [5].

In this study, a novel approach to generating NP-labeled DCs was introduced, and the influence of magnetosensitive complex on DCs antitumor activity was investigated. We hypothesized that increasing the targeted delivery of our elaborated DC-vaccine to the lymphoid tissue of mice through loading with MNC followed by exposition to the MF could enhance the efficacy of vaccine therapy in mice with Sa-37.

We took advantage of the fact that immature DCs naturally sample their surroundings and capture cellular debris as well as NP thus producing a magnetosensitive system for strengthening endogenous DCs trafficking after their inoculation [5]. In our study, MNC was internalized into DCs, and this was confirmed by Prussian blue staining. In the MF-treated group, the neodymium magnet was placed next to the inguinal lymph nodes of each mouse for 1 h, while MF was not applied to the mice of other groups. We registered a significant decrease in the volume and mass of the tumor in the mice that received the DCs loaded with MNC and MF. The greater antitumor effect of the magnetosensitive nanovaccine could be explained in terms of the improved targeted delivery of DCs to the lymph nodes controlled by the MF. Thus, the effectivity

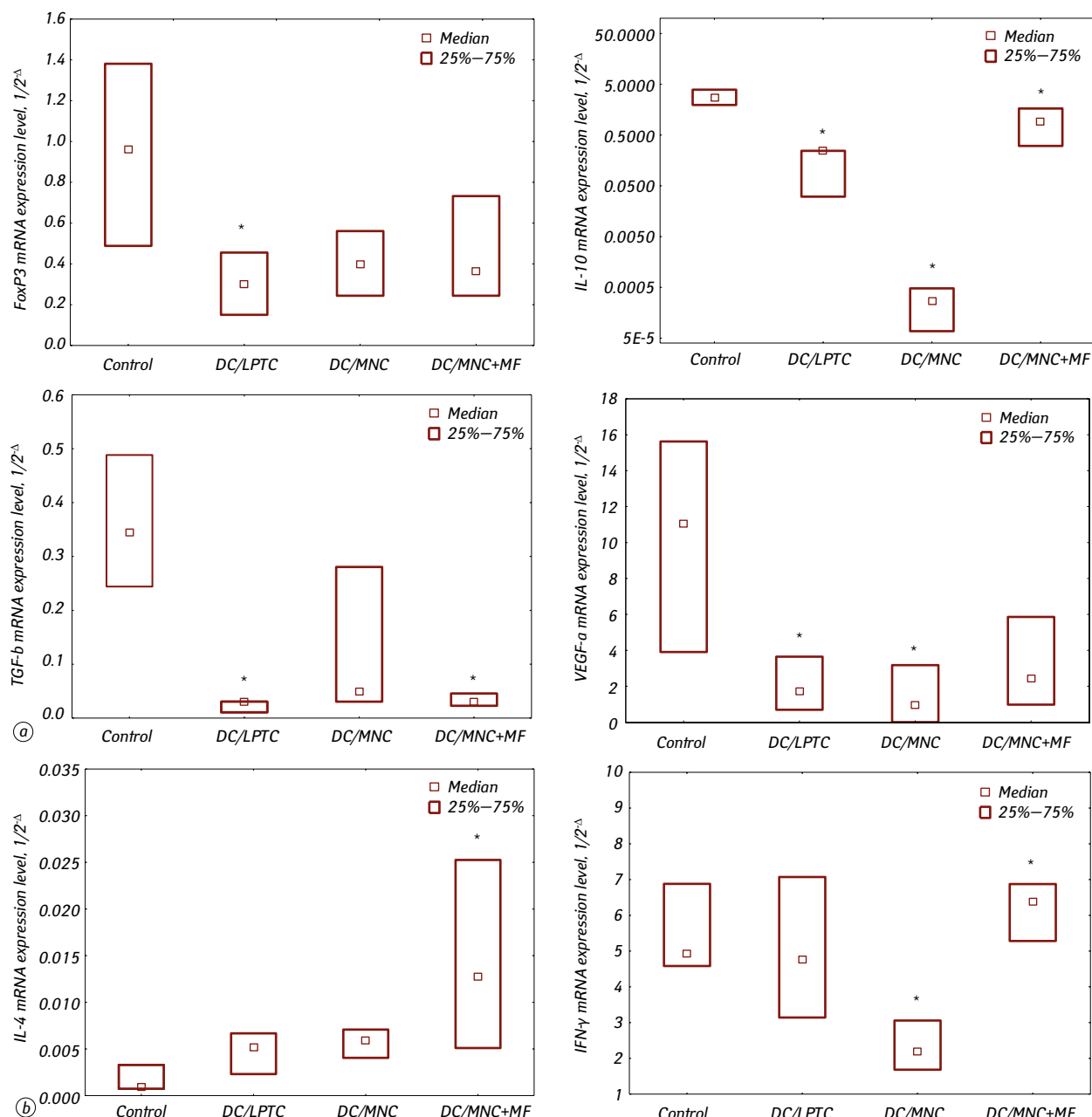


Fig. 4. Influence of MNC-loaded DCs on the mRNA expression level of cytokines, VEGF and nuclear factor FoxP3 in mice with Sa-37: *a* — mRNA expression level of cytokines with immunosuppressive properties, VEGF and nuclear factor FoxP3 in tumor tissue; *b* — mRNA expression level of IFN- γ and IL-4 in lymph node tissue. Data are presented as the Median (Q1 and Q3); * $p < 0.05$ in comparison with the control group of mice

of the antitumor nanovaccine based on DCs with tumor antigens and NP could be controlled by the constant MF. Our results are confirmed by the studies of several other authors. Jin *et al.* [4] showed that the locally applied MF can dramatically enhance the migration ability of DCs captured iron NP without altering their biodistribution pattern and thus enhance the antitumor immune response in tumor-bearing mice. However, it should be noted that in contrast to this work, in which DCs were injected into intact animals before tumor transplantation, we demonstrated the possibility of enhancing the antitumor activity of DC in tumor-bearing animals due to the correction of their down-regulated migration activity.

DCs present exogenous antigens on MHC class I molecules, thereby activating CD8⁺ T cells, contributing

to tumor elimination through a mechanism known as antigen cross-presentation [16]. Recently, the development of successful cancer immunotherapies may be attributed to the ability of DCs to cross-present tumor antigens [17, 18]. The mechanism of antigen internalization significantly influences the efficiency of cross-presentation; soluble antigens internalized by macropinocytosis are poorly presented, while particulate antigens, which enter cells via phagocytosis, are presented more efficiently by MHC class I molecules. More potent CTL responses are generated by particulate antigen delivery systems than those following the loading of soluble antigens. Therefore, various nanocarriers loaded with tumor antigenic material are currently being actively considered as potential factors involved in enhancing antigen

cross-presentation by DCs with a focus on cancer immunotherapeutics [19–21].

In this regard, we hypothesized that the loading of DCs with antigenic material as part of the MNC may enhance the cross-presentation of antigens in DCs. This, along with an increase in the delivery and presentation of antigen to T cells in the lymph nodes, this could contribute to a significant increase in the antitumor efficacy of the magnetosensitive DC nanovaccine. Moreover, in one publication, the authors hypothesize that NP engulfed as antigens by immature DCs induced early maturation of these DCs [4], which, in turn, may also contribute to their anticancer efficacy.

It is known that a variety of tumor-derived factors contribute to the emergence of complex local and regional immunosuppressive networks, including VEGF, IL-10, TGF- β , prostaglandin E2, and soluble phosphatidylserine, soluble Fas, soluble Fas ligand, and soluble MHC class I-related chain A proteins as well as the cells with immunosuppressive properties — Treg, M2 macrophages, myeloid-derived suppressor cell, tolerogenic DCs [9, 11, 22]. Tumor-derived factors drive the evolution of an immunosuppressive network, which ultimately extends immune evasion from the primary tumor site to peripheral sites in patients with cancer. TME can negatively affect the migration of endogenous DCs or DC vaccines to lymph nodes. Tumors recruit DCs precursors and may convert them into tolerogenic DCs that do not mature and this results in a lesser ability to migrate to lymph nodes [23–25]. It is widely accepted that, in particular, an immunosuppressive TME represents a major hurdle to cancer clearance by immune cells [26]. Therefore, targeting immunomodulatory pathways within the TME entered the center stage in cancer treatment [27, 28].

In our studies, it was demonstrated that any type of therapy — DCs loaded with LPTC, or MNCs with or without subsequent exposure to MP significantly contributed to a decrease in the mRNA expression level of immunosuppressive factors FoxP3, IL-10, TGF- β , and VEGF- α in tumor tissue. We can assume that DCs-based immunotherapy by itself may contribute to a decrease in the immunosuppressive properties of TME in mice with Sa-37. At the same time, the results showed that this mechanism alone is not sufficient for the implementation of the antitumor properties of the DC vaccine. Apparently, the balance between stimulatory and suppressive signals within the TME as well as systemic immunity should be investigated. Probably it is critical in dictating the ability of DCs-vaccine to induce and maintain an antitumor T cell response. Perhaps the lack of observed anti-tumor efficacy in the DC vaccine groups has simply been due to insufficient or inappropriate immune stimulation.

Naive T cell activation has long been known to be mediated by DCs within the draining lymph node [29]. We speculate that MNC and MF provide adjuvant activity by enhancing the delivery of tumor antigens to the draining lymph nodes and eliciting robust CD8⁺ T cell response. The evidence of the activation of the immune response in mice that received DCs loaded with MNCs under MF control is a significantly up-regulated expression

of pro-inflammatory cytokine IFN- γ in the inguinal lymph node tissue. Simultaneously, a significant up-regulation of the mRNA expression of the anti-inflammatory cytokine IL-4 was also revealed in the tissue of the draining lymph nodes. It should be noted that there was no increase in the expression of IL-4 and IFN- γ mRNA in the lymph node tissue of mice receiving DC vaccine as monotherapy. Thus, it can be concluded that the antitumor efficacy of the magnetically sensitive DC-nanovaccine is accompanied by the activation of the genes of the cytokines IL-4 and IFN in the tissue of the lymph node, which is regional in relation to the tumor.

Therefore, our results indicate the additional pathways that can be manipulated to increase DCs migration and tumor antigen presentation are needed to improve the anti-tumor efficacy of immunotherapy based on DCs. We have developed a technology for obtaining the MNC, which includes treatment of LPTC with NP of iron oxide Fe₂O₃ in a microvibromill. The magnetically sensitive antitumor vaccine based on mouse DCs and MNC has been developed. In experimental studies *in vivo*, we have shown that the maximum antitumor effect was achieved due to the use of DCs loaded with MNC under MF control. In conclusion, we suggest that the significant increase in the antitumor efficacy of DC vaccine can be achieved through the use of magnetosensitive nanocarriers under MF control.

CONFLICT OF INTEREST DISCLOSURES

All authors declare no conflict of interest.

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МАГНІТОЧУТЛИВИЙ НАНОКОМПЛЕКС ПОСИЛЮЄ ПРОТИПУХЛИННУ ЕФЕКТИВНІСТЬ ІМУНОТЕРАПІЇ ВАКЦИНАМИ НА ОСНОВІ ДЕНДРИТНИХ КЛІТИН

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Стан питання: Один з основних факторів недостатньої ефективності імунотерапії, яка ґрунтується на застосуванні дендритних клітин (ДК), полягає в малоефективній міграції цих клітин до лімфоїдної тканини, де ДК активують антиген-специфічні Т-клітини. Принципово новим підходом для посилення протипухлинних ефектів імунотерапії вакцинами на основі ДК може бути застосування наноконструкцій, чутливих до дії магнітного поля, для покращення таргетної доставки ДК до лімфатичних вузлів. **Мета:** Дослідити протипухлинні та імуномодуляторні ефекти ДК-нановакцини з магніточутливими властивостями та її вплив на імуносупресивне пухлинне мікрооточення на моделі саркоми 37 у мишей. **Матеріали та методи:** Досліджували протипухлинні, протиметастатичні та імуномодуляторні ефекти ДК, навантажених магнітним наноконструктом, спрямованих магнітним полем у мишей з перещепленою саркомою 37. **Результати:** Комбінована дія навантажених ДК та магнітного поля дозволяла суттєво зменшити об'єм та масу пухлини в порівнянні як з контролем, так і при застосуванні ДК без дії магнітного поля. Застосування ДК-нановакцини з магніточутливим наноконструктом як з дією магнітного поля, так і без нього супроводжується достовірним зниженням рівнів експресії мРНК FoxP3, трансформуючого фактора росту β, інтерлейкіну (ІЛ)-10 та фактора росту ендотелію судин у пухлинній тканині. У тварин, у яких застосовували ДК, навантажених магнітним наноконструктом, спрямованих магнітним полем, відзначали збільшення експресії мРНК інтерферон-γ та ІЛ-4. **Висновки:** Застосування ДК з магніточутливими наноконструктами, спрямованих магнітним полем, дозволяє посилити протипухлинну ефективність ДК вакцини.

Ключові слова: дендритні клітини, наночастинки оксиду заліза, мишача саркома, протипухлинний ефект.