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CRYOPRESERVATION REGIMENS OF BONE MARROW MONONUCLEAR CELLS DETERMINE THE FORMATION OF IMMUNE DENDRITIC CELLS

One of the innovative directions in the therapy of oncology diseases is the use of vaccines based on immune dendritic cells (iDCs). The paper presents the results of obtaining in vitro iDCs from bone marrow mononuclear cells (MNCs) cryopreserved under different regimens using cryolysate of Ehrlich ascites carcinoma a cells. For the formation of iDCs, granulocyte-macrophage colony-stimulating factor, interleukin-4 and cryolysate of Ehrlich ascites carcinoma cells were added to the culture medium as an inducer of dendritic cells (DCs) maturation. It was proven that immature DCs obtained from native or cryopreserved MNCs responded differently to the addition of inducers of iDCs formation. The cryolysate turned out to be a more potent inducer of iDCs than lipopolysaccharide, which was manifested by an increase in the expression level of all studied markers (CD11c, CD80, CD83, CD86). At the same time, the cryolysate maximally stimulated the expression of maturity markers (CD11c, CD83) on iDCs, which were formed from cryopreserved MNCs using regimens 1 at a rate of 1 deg / min to –80 °C with subsequent immersion in liquid nitrogen. Our findings demonstrate the possibility of obtaining in vitro iDCs from MNCs cryopreserved under certain conditions using cryolysate of Ehrlich adenocarcinoma cells for further use in immunotherapy of oncology pathology.

Keywords: *cryopreservation, bone marrow mononuclear cells, immune dendritic cells, cells cryolysate, Ehrlich ascites carcinoma.*

MHC II — major histocompatibility complex class II
GM-CSF — granulocyte-macrophage colony-stimulating factor
DCs — dendritic cells
DMSO — dimethyl sulfoxide
FCS — fetal calf serum
iDCs — immune dendritic cells
IL — interleukin
CL — cryolysate
BM — bone marrow
CryoDC — dendritic cells derived from cryopreserved mononuclear cells
CryoMNCs — cryopreserved mononuclear cells
LPS — lipopolysaccharide
MNCs — mononuclear cells
NatDCs — dendritic cells derived from native mononuclear cells
NatMNCs — native mononuclear cells
R1, R2 — cryopreservation regimens 1, 2
Hsp70 — heat shock protein with a molecular weight of 70 kDa
NF-κB — nuclear factor-kappa B
PI — propidium iodide
TLR-4 — toll-like receptor-4

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Treatment of oncology pathologies remains an urgent challenge in medicine. Today, a clear trend is being determined to expand the spectrum of methods of treating cancer patients based on data on the interaction of tumor cells with the immune system of tumor-bearing [26, 34, 41]. The results of studies assessing the therapeutic success of vaccines based on immune dendritic cells (iDCs) have shown their possibility of inducing a specific immune response to tumor antigens in various types of cancer [7, 15, 37]. The antitumor immune response of dendritic cells (DCs) is implemented through the absorption, processing and presentation of antigens on molecules of the major histocompatibility complex class II (MHC II), which ensures the maturation and activation of DCs. As a result, DCs have a reduced ability to phagocytosis, increased expression of major histocompatibility complex (MHC) II and costimulatory molecules, and increased production of proinflammatory cytokines required for T cell activation [11, 17]. To initiate the activation of naive T cells chemokine receptor 7 (CCR7) is expresed on the iDCs membrane, duets which the latter transport tumor antigens to the lymph nodes. The essential mechanisms of the antitumor action of iDCs are the recruitment of T cells into the tumor microenvironment by chemotactic factors produced by DCs and their subsequent interaction *in situ* with effector T cells [26]. The unique ability of iDCs to activate $CD4^+$ T helper and $CD8^+$ cytotoxic T lymphocytes initiates an adaptive immune response [11]. This fact is crucial for designing the antitumor vaccines. In this case, the use of both bone marrow (BM) mononuclear cells (MNCs) and peripheral or cord blood monocytes as a source of iDCs precursors is envisaged [7, 10, 42].

A common way for obtaining iDCs *ex vivo* is a two-stage stimulation of MNCs [37]. At the first stage, immature DCs are obtained from BM or peripheral blood MNCs by culturing with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) [18, 21]. In the second stage, immature DCs are "loaded" with tumor antigens, which determine the specificity of the antitumor effect of the obtained iDCs [30].

Autologous tumor cells are considered an effective source of tumor antigens for iDC-based vaccine therapy [9]. Tumor cells contain a full set of individual tumor-associated antigens of the tumor-bearing), which are crucial for activating the immune response and destroying the tumor [8]. The wide antigenic "repertoire" of tumor cell lysates obtained by various methods, including the process of multiple freeze-thaw, ensures balanced activation of all components of the tumor-bearing immune system, particularly the formation of cytotoxic effector cells, as well as humoral factors of antitumor defence [2]. More than 50 years ago, the first reports appeared on the effect of ultralow temperatures on tumor tissues, modification of their structure and special ability to activate the antitumor immune response [1]. In addition to the ability to increase the immunogenic properties of cells, ultralow temperatures are widely used for long-term storage of biomaterial. Cryopreservation is known to be an integral stage of the biotechnological process before the clinical application of iDCs. To successfully derive the iDCs from cryopreserved progenitor cells, their slow freezing with rapid warming has been proposed [4, 17, 28, 33, 36]. Traditionally, fetal bovine serum (FBS) at a concentration of 20–90 % is a mandatory component of the cryomedium for freezing MNCs [17, 19]. However, as G. F. Silveira *et al.* reported [36], the DCs derived from cryopreserved peripheral blood mononytes retain an immature phenotype with limited maturation potential in the presence of inducers, in particular lipopolysaccharide (LPS). This may be due to the inhibition of expression on the formed DCs of receptors for GM-CSF (CD116) and IL-4 (CD124) along with a low level of expression of toll-like receptor-4 (TLR-4), which accept LPS [27, 40]. In view of the above, the issues of developing and testing experimentally substantiated cryopreservation regimens for MNCs with subsequent evaluation of iDCs derived from them remain important.

The aim of this research was to study the possibility of obtaining immune dendritic cells *in vitro* from bone marrow mononuclear cells cryopreserved under different regimens.

MATERIALS AND METHODS

The experiments were performed in 6-month-old female BALB/c mice, which were kept under standard conditions at the Animals House Facility of the Institute for Problems of Cryobiology and Cryomedicine of the National Aca-

demy of Sciences of Ukraine (IPCC of the NAS of Ukraine, Kharkiv). The research was performed in accordance with the requirements of the Bioethics Committee of the IPCC of the NAS of Ukraine in accordance with the Law of Ukraine "On the Protection of Animals Against Cruelty" (3447-IV of 21.02.2006) and the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Deriving the bone marrow cells. Bone marrow was aspirated from mouse femurs and resuspended with RPMI-1640 (Biowest, France) supplemented with 3 % ETC (Biowest) and 2 % sodium citrate (hereinafter referred to as handing medium) and passed through a nylon filter with a pore diameter of 100 μm (Falkon, USA), centrifuged (200g, 10 min) and the resulting precipitate was resuspended in handling medium.

Isolation of mononuclear cells from bone marrow. Mononuclear cells were isolated by centrifugation of the bone marrow suspension in a density gradient (1.077 g / ml) of the drug "Trazograf" (Unique Pharmaceutical Laboratories, India) [12].

Cryopreservation of mononuclear cells. For cryopreservation of MNCs, a solution consisting of RPMI-1640 medium with 20 % FBS and 20 % dimethyl sulfoxide (DMSO) (Arterium, Ukraine) was used. To the MNCs suspension (1×10^7 cells/ml), a cryopreservation solution was added dropwise in a ratio of 1:1 with a final cryoprotectant concentration of 10 %. The MNCs suspension was placed in 1.8 ml plastic ampoules (Nunc, Germany). The cells in this cryopreservation solution were exposed for 10 min at room temperature (20–24) °C.

In the presented study, the MNCs were cryopreserved in two regimens. Regimen 1 (R1) was carried out using the "Mr. Frosty™" (Thermo Scientific, USA) at a rate of about 1 deg/min to –80 °C and subsequent immersion in liquid nitrogen (–196 °C). Regimen 2 (R2) was performed using a ZP-10 programmable freezer (Special Constructing and Designing Unit of the IPCC of the National Academy of Sciences of Ukraine) at a rate of 1 deg / min to –40 °C and subsequent immersion in liquid nitrogen (–196 °C). The choice of these cryopreservation regimens was justified by the following: R1 has a wider application during cryopreservation of MNCs or human peripheral blood monocytes to obtain iDCs for clinical use [4] and high efficiency during cryopreservation of cell spheroids, which is proven by the results of physical and mathematical modelling of mass transfer [29]; R2 allows passing the eutectic crystallization point of a 10 % DMSO solution (–66.7 °C) with a higher cooling rate (from –40 to –196 °C), which reduces a cell cryodestruction during freeze-thawing [16]. The effectiveness of R2 in obtaining immature DCs was proven by the research results of H. Kysielova *et al.* [24]. In our study, the possibility of obtaining iDCs from cryopreserved MNCs using R1 and R2 was comparatively assessed.

The samples were warmed in a water bath at 40 °C until the solid phase disappeared. The cells were washed once from DMSO by slowly adding a double volume of handling medium and subsequent centrifugation (200 g, 10 min). The number of MNCs was counted in a Goryaev chamber.

Assessment of structural and functional characteristics of mononuclear cells. MNCs viability was determined using propidium iodide – PI (Becton Dickinson, USA) on a flow cytometer "FACS Calibur" (Becton Dickinson) according to the manufacturer's instructions. Propidium iodide is a membrane-impermeable dye for intact cells. In case of disruption of the cell membrane integrity, it enters the cell and binds to DNA. Propidium iodide is usually used to detect non-viable cells (PI^+ cells) at the stage of necrosis or late apoptosis.

The number of viable MNCs was determined by the formula:

$$\text{Viability} = 100\ \% - \text{number of PI + cells.}$$

$CD14^+$ cells in the MNCs were phenotypically assessed with a flow cytometer "FACS Calibur" (Becton Dickinson) using monoclonal antibodies CD14 FITC (Becton Dickinson) according to the manufacturer's instructions.

In vitro production of immune dendritic cells. MNCs were cultured in RPMI-1640 medium supplemented with 10 % FBS and 1 % antibiotic solution (100 units/ml penicillin, 0.1 mg/ml streptomycin) in 3 cm diameter plastic Petri dishes (plating dose 1×10^7 cells/dish) [31]. Cells were cultured at 37 °C in an atmosphere of 5 % CO_2. After 2 hours, the medium with non-adherent cells was removed and 3 ml of fresh culture medium containing GM-CSF (20 ng/ml) and IL-4 (5 ng/ml) (Sigma-Aldrich, United Kingdom) was added. The medium was added on the 1st and 3rd culturing days. On the 5th day of cultivation, half of the cul-

ture medium was removed from the dishes and 3 ml of fresh culture medium with GM-CSF (20 ng/ml), IL-4 (5 ng/ml) and cryolysate (CL) of tumor cells (1.0 mg protein/ml) was added. As a control, 3 ml of fresh culture medium with 100 ng/ml LPS (O26:B6, Escherichia coli, Sigma-Aldrich) was added to the dishes.

We emphasize that we obtained DCs from native mononuclear cells (NatMNCs) or cryopreserved mononuclear cells (CryoMNCs), which will be referred to as NatDCs or CryoDCs in the following. Cells obtained *in vitro* from CryoMNCs with R1 (CryoR1MNCs) or R2 (CryoR2MNCs) are designated as CryoR1DCs or CryoR2DCs, respectively.

The following experimental groups were formed:

group 1 — immature NatDCs obtained from NatMNCs when adding GM-CSF and IL-4 to the culture medium, $n = 5$;

group 2 — immune NatDCs obtained from NatMNCs when adding GM-CSF, IL-4 and CL to the culture medium, $n = 5$;

group 3 — immune NatDCs obtained from NatMNCs when adding GM-CSF, IL-4 and LPS to the culture medium, $n = 5$;

group 4 — immature CryoR1DCs obtained from CryoR1MNCs when adding GM-CSF and IL-4 to the culture medium, $n = 5$;

group 5 — immature CryoR2DCs obtained from CryoR2MNCs when adding GM-CSF and IL-4 to the culture medium, $n = 5$;

group 6 — immune CryoR1DCs obtained from CryoR1MNCs when adding GM-CSF, IL-4 and CL to the culture medium, $n = 5$;

group 7 — immune CryoR2DCs obtained from CryoR2MNCs when adding GM-CSF, IL-4 and CL to the culture medium, $n = 5$;

group 8 — immune CryoR1DCs obtained from CryoR1MNCs when adding GM-CSF, IL-4 and LPS to the culture medium, $n = 5$;

group 9 — immune CryoR2DCs obtained from CryoR2MNCs when adding GM-CSF, IL-4 and LPS to the culture medium, $n = 5$.

On day 7 of MNCs cultivation, DCs were obtained, they were removed with a scraper and subjected to two centrifugations (200g, 10 min) in 5 ml of phosphate-buffered saline (Sigma-Aldrich, Germany). At all stages of cultivation, cell growth was monitored using an inverted microscope "Axiovert 40C" (Carl Zeizz, Germany).

Assessment of structure and functions of immune dendritic cells. The number of cells was counted in a Goryaev chamber, their viability was assessed with a flow cytometer "FACS Calibur" (Becton Dickinson) using PI.

Phenotypic characteristics of cells obtained *in vitro* on the 7th day of cultivation were determined by means of a flow cytometer "FACS Calibur" (Becton Dickinson) using monoclonal antibodies CD14 FITC (Becton Dickinson), CD83-PE (Becton Dickinson), CD11c-FITC (Becton Dickinson), CD80-FITC (Biolegend, USA), CD86-FITC (Biolegend) and corresponding isotype controls according to the manufacturer's instructions. For each sample, 10,000 flow cytometer events were analyzed using the software "CellQuest Pro" (BD Biosciences, USA). The findings were analysed with "WinMDI 2.8" (Joseph Trotter, USA).

Tumor cell cryolysate preparing. Ehrlich ascites carcinoma cell cryolysate was a source of tumor antigens for obtaining iDCs [12]. Tumor cells at a concentration of 15.0×10^6 cells/ml were frozen five times in succession (to –196 °C) without cryoprotectant, thawed for 20 min at room temperature (20—24 °C) with constant shaking of the ampoules until the solid phase disappeared. Total protein in tumor cell CL was determined by the colorimetric biuret method using the "Total Protein" kit (Filisit-Diagnostics, Ukraine). The total protein concentration in the obtained CL was 14.0 mg/ml. Tumor cell CL was placed in cryotubes (1 ml) and stored at –20 °C until use.

Statistical data processing was performed using Microsoft Excel 2010 spreadsheets and the Statistics 10.0 software (StatSoft, USA). Quantitative data were processed using the variational statistics method with the calculation of the median (Me) and interquartile range [25; 75]. Independent samples with the control group were compared using the Mann-Whitney method. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that cryopreservation of MNCs under different regimens did not significantly affect their viability determined using PI (R1 — 89.0 % and R2 — 87.0 %, respectively) compared to NatMNCs (96.0 %). The R1 and R2 regimens also ensured the maintenance of almost the same high number

of MNCs (8.6×10^6 and 7.8×10^6 cells/ml, respectively) compared to NatMNCs ($10.6 \times \times 10^6$ cells/ml).

It is meaningful that after cryopreservation of MNCs, the concentration of $CD14^+$ cells in them increased compared with NatMNCs. Thus, when using the R1 and R2, this marker was expressed by 38.0 and 36.8 % of cells, respectively.

The CD14 molecule is known to be a membrane glycoprotein co-receptor in the CD14/TLR4/MD2 cellular receptor complex, which recognizes antigenic stimuli, including bacterial LPS [43]. The obtained data indicate that among NatMNCs, 29.1 % of cells expressed the CD14 marker (Table 1), and in CryoMNCs this index tended to increase, and mostly when using R1. It has been proven that cryopreservation can affect the differentiation of MNCs into DCs with subsequent changes in the response of formed DCs to inflammatory stimuli or pathogens [24, 28, 36].

Particular attention in changing the functional state of DCs during their differentiation from immature to immune DCs is focused on heat shock proteins (HSPs) of the Hsp70 family, which regulate adaptive immune system responses and control the proliferation of immune competent cells [25]. It is known that Hsp70 proteins can influence the expression of nuclear factor-kappa B (NF-κB) as the inflammation main trigger. An increased concentration of intracellular Hsp70 blocks the activation of NF-κB and causes the formation of tolerogenic DCs and, as a result, the production of the anti-inflammatory cytokine IL-10. Such DCs are used in immune suppressive therapy of autoimmune diseases [6, 24]. The opposite effect occurs when Hsp70 is extracellular and can stimulate the immune response by increasing the production of NF-κB-dependent proinflammatory cytokines (IL-6, IL-1β and TNF-α), which activates inflammation [20]. Therefore, the mechanism of action of Hsp70 is implemented indirectly by modulating the phenotype and function of both bone marrow precursors and DCs derived from them [39].

Table 2 shows that immature NatDCs generated from NatMNCs (group 1) and immune NatDCs obtained in the presence of maturation activators as tumor cells cryolysate (group 2) or LPS (group 3) had high viability (89—91 %). It is significant that the maximum number of immune NatDCs (72.5 % of the initial number of cells plated on a dish) was formed in group 2 after the addition of cryolysate to the culture medium, which confirms its more potent immunogenicity compared to LPS. After culturing CryoR1MNCs and CryoR2MNCs (groups 4 and 5) without maturation inducers, both viability and DCs number were lower than those of group 1 (NatDCs).

However, after culturing CryoR1MNCs in the presence of cryolysate (group 6), the largest number of immune CryoR1DCs (66.7 % of the initial number of cells plated on a dish) with a viability of 90% was obtained. After the addition of LPS to the Cryo-MNCs culture (groups 8 and 9), the viability of the obtained iDCs did not differ from that after adding the cryolysate (groups 6 and 7), but their number was lower. It seems that the ability to mature iDCs is realized differently in native or cryopreserved MNCs, *i. e.* the initial state of the MNCs is important. This postulate is influential for confirming the presence of certain characteristic molecules on the surface of iDCs, which determine their maturity extent by phenotypic signs in the course of formation in a culture with MNCs [38].

A well-studied surface marker of iDCs is the CD83 glycoprotein, which does not belong to

Table 1. **Indices characterizing the state of bone marrow MNCs after cryoprervation**

Type of material	Показник		
	MNCs viability, %	MNCs number, $\times 10^6$ cells / ml	$CD14^+$ cell concentration among MNCs, %
NatMNCs	96.0 [95.0; 98.0]	10.6 [8.7; 11.3]	29.1 [26.4; 31.4]
CryoR1MNCs	89.0 [85.0; 96.0]	8.6 [7.1; 9.8]	38.0 [36.2; 39.6]*
CryoR2MNCs	87.0 [86.0; 97.0]	7.8 [6.5; 8.7]	36.8 [34.5; 38.4]*

* Difference is significant in comparison with similar indices of NatMNCs, $p < 0.05$

the IgG superfamily of immunoglobulins. It consists of extracellular Ig-like, transmembrane and intracellular domains of 39 amino acids. W. Cao *et al.* [5] reported that the CD83 structure is a preformed protein inside monocytes, macrophages and immature DCs, and its expression on the membrane surface is induced during DCs activation. It is important to note that monocytes and macrophages only transiently express this marker, whereas mature iDCs stably and densely express the CD83 structure [5]. Immune DCs also express the surface membrane marker CD11c [23]. This protein is specific to myeloid DCs and forms a complex with CD18 (integrin β-2 chain) that is a receptor for the complement component iC3b and is also involved in cell adhesion. However, CD11c can be expressed on NK cells and populations of activated T and B cells [32]. Another essential membrane molecule of mature DCs is the costimulatory molecule B7 (CD80/CD86), which belongs to the immunoglobulin superfamily [22]. It is important that each of the above-mentioned membrane structures of iDCs plays a certain role in implementing the multi-profile and multi-stage structural and functional potential of iDCs [41]. There is no doubt that the quantitative and qualitative characteristics of these indices may change when iDCs are obtained from MNCs that have been exposed to ultralow temperatures and subsequently exposed to various forms of stimuli added as the ones for the maturation and formation of iDCs only [17, 27, 28, 32].

In the presented study, the affiliation of DCs to immune cells was identified by markers CD14/CD83, CD11c, CD80, CD86. As can be seen from the figure, among immature NatDCs formed from NatMNCs without the addition of cryolysate or LPS, only a small percentage (6.9 [4.7; 8.6] %) expressed $CD14^{-}CD83^{+}$ markers (Figure, A). A significantly higher percentage was $CD11c^{+}$-DCs (34.1 [31.3; 35.8] %) (Figure, B), as well as cells expressing the costimulatory molecules CD80 and CD86 (36.1 [32.5; 39.7] % and 45.6 [40.9; 47.8] %, respectively) (Figure C, D).

It is noteworthy that the formation of the number of $CD80^{+}$- or $CD86^{+}$-DCs depends on the cryopreservation conditions of the MNCs which they were derived from (Figure C, D). Cryopreservation

Table 2. **Indices of DCs obtained *in vitro* on the 7th day of cultivation from native or cryopreserved MNCs**

Group number	Index		
	DCs viability. %	DCs number	
		% of MNCs plated on a plate	absolute. × 10^6
1	91.0 [86; 95]	52.5 [49.3; 53.4]	5.2 [4.9;5.3]
2	90.0 [84; 95]	72.5 [70.1; 74.4]*	7.3 [7.0; 7.4] *
3	89.0 [85; 94]	55.7 [53.2; 56.8]	5.6 [5.3; 5.7]
4#	84.0 [82; 87]	45.6 [45.1; 48.4]*	4.5 [4.5; 4.8]*
5&	81.0 [76; 86]	41.6 [41.0; 43.8]*	4.2 [4.1; 4.4]*
6#	90.0 [90; 91]	66.7 [64.7; 68.3]*	6.7 [6.5; 6.8]*
7&	87.0 [85; 88]	51.3 [45.5; 55.9]	5.1 [4.6; 5.6]
8#	82.0 [78; 86]	50.0 [48.4; 51.1]	5.0 [4.8; 5.1]
9&	81.0 [77; 86]	46.0 [44.6; 48.0]*	4.6 [4.5; 4.8]*

— DCs obtained from CryoR1MNCs, & — DCs obtained from CryoR2MNCs; *— difference is significant compared with similar indices of group 1 (DCs obtained from NatMNCs), $p < 0.05$

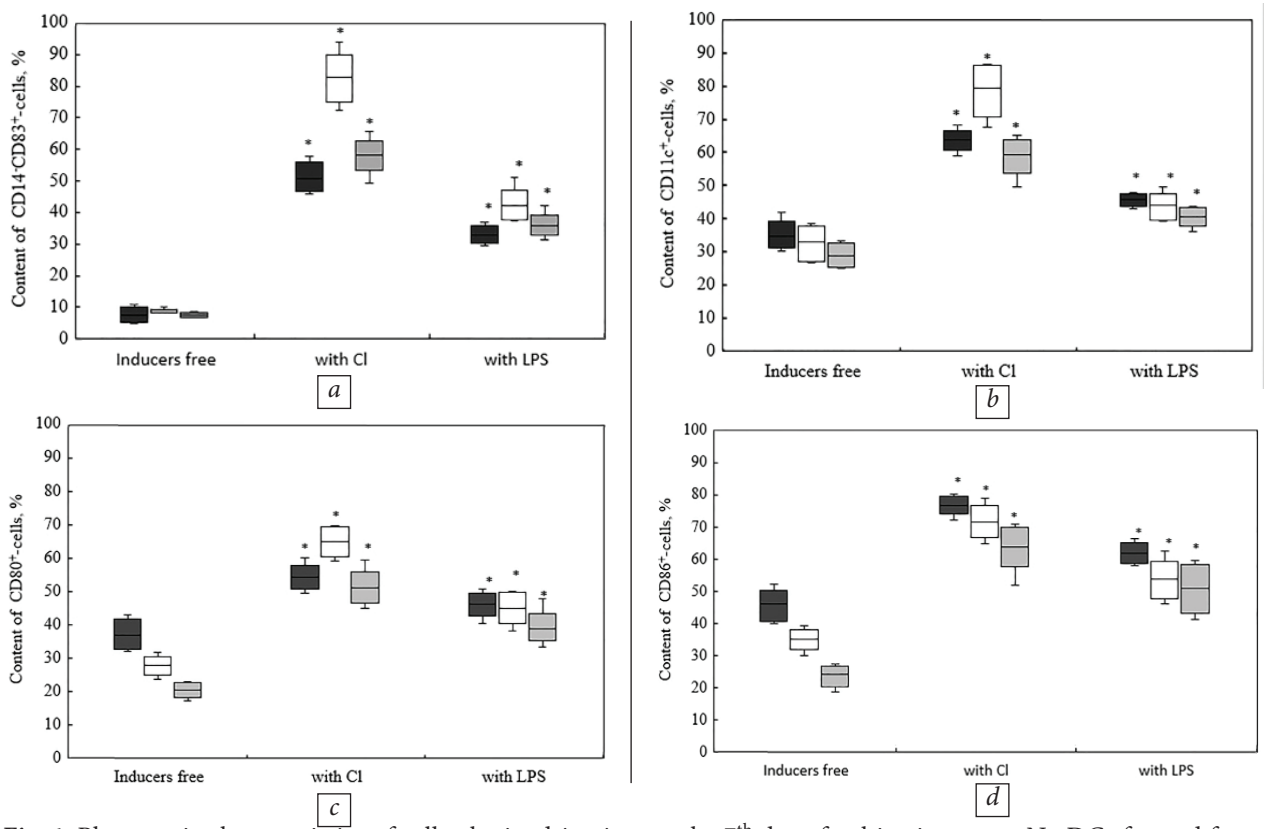

Fig. 1. Phenotypic characteristics of cells obtained *in vitro* on the 7th day of cultivation: ■ — NatDCs formed from NatMNCs; □ — CryoR1DCs formed from CryoR1MNCs;; ■ — CryoR2DCs formed from CryoR2MNCs; by markers CD83 (*a*), CD11c (*b*), CD80 (*c*), CD86 (*d*), $n = 5$. * — difference is significant in comparison with similar indices of NatDCs or CryoDCs without maturation inducers, $p < 0.05$

generally inhibited the ability of MNCs to form CryoDCs with $CD80^+$ and $CD86^+$ phenotypes, and to a greater extent when using R2. This may be due to an increase in the content of intracellular Hsp70 in DCs obtained from MNCs cryopreserved at R2. It is known that Hsp70 in DCs suppresses the expression of CD86, MHC II and inhibits the production of TNF-α, which leads to their production of the anti-inflammatory cytokine IL-10 and, consequently, to immune suppression [3, 24]. Cryopreservation can cause shedding of certain receptors on cell membranes, which disrupts the intercellular communication links of immune competent cells and, as a result, reduces their function [14]. Studies of membrane receptors on immature CryoDCs formed from CryoMNCs without maturation inducers showed that cryopreservation conditions inhibit the expression of costimulatory molecules (CD80, CD86) more than maturity markers (CD11c, CD83) on DCs.

It is significant that DCs obtained from native or cryopreserved precursors responded differently to iDC formation inducers. At the same time, cryolysate maximally stimulated the expression of maturity markers (CD11c, CD83) on CryoR1DCs. In other words, cryopreservation of MNCs with R1 can booster the formation of mature iDCs in response to cryolysate. In addition, cryolysate turned out to be a more powerful inducer of iDCs compared to LPS, which is confirmed by their phenotypic characteristics.

Thus, our findings demonstrate the possibility of obtaining immune CryoDCs using cryolysate of Ehrlich ascites carcinoma cells. Their formation depends on the conditions of cryopreservation and *in vitro* cultivation of MNCs from which they are obtained. This fact must be assumed to be further used during immune correction of oncology pathology.

CONCLUSIONS

Thus, based on the obtained results we have found:

1. The iDCs were derived from bone marrow MNCs cryopreserved under different regimens after adding to the culture medium, in addition to

GM-CSF and IL-4, cryolysate of Ehrlich ascites carcinoma cells or lipopolysaccharide.

2. The use of cryolysate, unlike lipopolysaccharide, stimulated the appearance of iDCs with characteristic markers of maturity (CD83, CD11c) and costimulatory molecules (CD80, CD86). At the same time, the cryolysate maximally stimulated the expression of maturity markers on immune CryoR1DCs, which were formed from cryopreserved MNCs under regimen 1 at a rate of 1 deg/min to –80 °C with subsequent immersion in liquid nitrogen.

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РЕЖИМИ КРІОКОНСЕРВУВАННЯ МОНОНУКЛЕАРІВ КІСТКОВОГО МОЗКУ ОБУМОВЛЮЮТЬ ФОРМУВАННЯ ІМУННИХ ДЕНДРИТНИХ КЛІТИН

Одним з інноваційних напрямків у терапії онкологічних захворювань є використання вакцин на основі імунних дендритних клітин (іДК). У роботі представлено результати отримання in vitro іДК із кріоконсервованих за різними режимами мононуклеарів (МНК) кісткового мозку з застосуванням кріолізату клітин аденокарциноми Ерліха. Для формування іДК у середовище культивування вносили гранулоцитарно-макрофагальний колоніє-стимулюючий фактор, інтерлейкін-4 та кріолізат клітин аденокарциноми Ерліха як індуктора созрівання дендритних клітин (ДК). Доведено, що незрілі ДК, отримані з нативних або кріоконсервованих МНК, по-різному відповідали на додавання індукторів формування іДК. Кріолізат виявився більш потужним індуктором іДК, ніж ліпополісарид, що маніфестувалося підвищенням рівня експресії усіх досліджуваних маркерів (CD83, CD11c, CD80, CD86). При цьому кріолізат максимально стимулював експресію маркерів зрілості (CD11c, CD83) на іДК, які були сформовані з кріоконсервованих МНК за режимом 1 зі швидкістю 1 град / хв до –80°С з подальшим зануренням у рідкий азот. Одержані результати свідчать про можливість отримання in vitro іДК із кріоконсервованих за певних умов МНК з використанням кріолізату клітин аденокарциноми Ерліха для подальшого застосування в імунотерапії онкопатології.

Ключові слова: кріоконсервування, мононуклеари кісткового мозку, імунні дендритні клітини, кріолізат пухлинних клітин, аденокарцинома Ерліха.