Աղյուսակ 1. ԴԲՄ-ի տեսակարար ակտիվությունը ինսուլին-կախյալ շաքարախտով հիվանդների և առողջ մարդկանց արյան շիճուկում

Ցուցանիշ	Առողջներ	Հիվանդներ
Ուսումնասիրված անձանց թիվը	42	11
ԴԲՄ-ի միջին տեսակարար ակտիվություն; M±m	22,7±2,0	4,9±0,9
ԴԲՄ-ի նվազագույն տեսակարար ակտիվություն	3.5	0.9
ԴԲՄ-ի առավելագույն տեսակարար ակտիվություն	45.7	9.4

ՇՆՈՐՀԱԿԱԼԱԳԻՐ: Հեղինակը շնորհակալություն է հայտնում իր ղեկավարին՝ ՀՀ ԳԱԱ մոլեկուլային կենսաբանության մակրոմոլեկուլային կոմպլեքսների լաբորատորիայի վարիչ, կենս. գ. դ-ր Ա.Բոյաջյանին, լաբորատորիայի գիտական աշխատակից կենս. գ. թեկն. Կ. Մայիլյանին, «Արմենիա» հանրապետական բժշկական կենտրոնի ներքին հիվանդությունների բաժանմունքի վարիչ պրոֆ. Վ. Հարությունյանին:

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COMPOSITION OF PHOSPHOLIPIDS AND GANGLIOSIDES IN LYMPHOCYTES DURING DIFFERENTIATION IN NORMAL AND TUMOR–BEARING ANIMALS

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Phospholipid and ganglioside composition in bone marrow progenitors of lymphocytes, thymocytes, and mature lymphocytes of intact rats and rats with sarcoma 45 were studied. Alterations in lipids spectrum (both phospholipids and gangliosides) were shown to take place during lymphocyte differentiation of intact animals. The exchange of phospholipid composition in tumor-bearing animals in process of lymphocyte maturation was shown. The rate of ganglioside sialylation was diminished in rats with sarcoma 45. Tumor-induced alterations in lymphocyte lipid composition involve all stages of lymphocyte differentiation. These shifts are believed to be connected with a disturbance of antineoplastic function of lymphocytes and, consequently, disorders of lymphocyte maturation, and inhibiting of the immune response of the tumor-bearing organism.

Զաքարյան Ա., **Առողջ և ուռուցքակիր առնետների տարբերակման պրոցեսում գտնվող լիմֆոցիտների ֆոսֆոլիպիդային և գանգլիոզիդային կազմը:** Հետազոտվել է առողջ և սարկոմա-45 ուռուցքակիր առնետների ոսկրածուծի բնային բջիջների, թիմոցիտների և պերիֆերիկ արյուն հասուն լիմֆոցիտների ֆոսֆոլիպիդային և գանգլիոզիդային կազմը: Ցույց է տրվել, որ առողջ կենդանիների մոտ տարբերակվող լիմֆոցիտներում ընկատվում են ֆոսֆոլիպիդային և գանգլիոզիդային կազմի փոփոխություններ: Ուռուցքակիր-առնետների լիմֆոցիտների հասունացման պրոցեսի բոլոր փուլերում բացահայտվել են վերոհիշյալ նյութերի բաղադրակազմի փոփոխությունների ակնհայտ տարբերություններ: Սարկոմա-45 ուռուցքակիր առնետների լիմֆոցիտներում հայտնաբերվել է գանգլիոզիդների սիալացման մակարդակի կտրուկ անկում: Ցույց է տրվել, որ լիպիդային կազմի նշված փոփոխությունները հետևանք են չարորակ նորագոյացության զարգացման և համապատասխանում են լիմֆոցիտների հակաուռուցքային ֆունկցիայի խանգարմանը:

Закарян А. **Спектр фосфолипидов и ганглиозидов в лимфоцитах в процессе дифференцировки в норме и у животных опухоленосителей.** Впервые охарактеризован состав фосфолипидов и ганглиозидов в костномозговых предшественниках лимфоцитов, тимоцитах и зрелых лимфоцитах периферической крови у интактных крыс и крыс с саркомой 45. Показано, что происходит изменение фосфолипидного и ганглиозидного состава в период созревания лимфоцитов у здоровых животных. У крыс опухоленосителей наблюдалось выраженное изменение спектра фосфолипидов на всех стадиях дифференцировки лимфоцитов. Обнаружено снижение уровня сиалирования ганглиозидов у крыс с саркомой 45. Выявленные изменения липидного состава происходят под действием развивающейся опухоли и затрагивают все стадии дифференцировки лимфоцитов. Эти изменения коррелируют с нарушением противоопухолевой функции лимфоцитов вследствие расстройства их созревания и подавления иммунного ответа у животных опухоленосителей.

INTRODUCTION. Cell membrane lipids are known to be involved in cell differentiation [1-3]. Phospholipids of the cell membrane take part in processes of lymphocyte maturation from bone marrow progenitors to mature functional cells of peripheral immune system [4-6]. It was demonstrated earlier [7] that changes in fatty acid composition of membrane phospholipids alter properties and some cell functions including immune cell reactions. In several types of cancers different alterations in phospholipids pattern were observed [8, 9]. Modifications in phospholipid spectrum were described also in non-cancer cells of organisms affected by cancer [10, 11].

Gangliosides produced by tumor cells were shown to shed into pericellular space [12, 13] and to bind host immune cells [14] inhibiting the cellular immune response [15]. So, cell lipid composition and functions seem to be involved in malignization process. It is interesting to follow changes in phospholipid and ganglioside (in lymphocytes related to ganglioseries) composition of immune cells during their maturation and alterations of these lipids under influence of solid tumor developing in the organism.

MATERIALS AND METHODS. Number of animals used (120-150*g* weight) was 16. They were separated into two groups. The 1st groups (8 rats) of intact animals were used as a control. Rats of the 2nd group were grafted with sarcoma 45 by subcutaneous injection of 1 x 10^6 tumor cells suspended in 0,2*ml* saline into right side of thorax. The grafting success rate was shown to be about 95%. Animals that had well-expressed tumor after 14 days were used.

Bone marrow cells were obtained by washing of femurs with cold Hanks' solution. Thymus tissue was homogenized in the same solution. The bone marrow lymphocyte progenitors and thymocytes were isolated by Ficoll-Paque (1,07) density centrifugation (1500 *rpm*, 40 min.). The blood was diluted by Hanks' solution (1:1). Mature lymphocytes were isolated by Ficoll-Paque (1,87) density centrifugation (1500 *rpm*, 40 min.) (12). The cells obtained were washed two times with Hanks' solution. Macrophages were removed by differential adhesion technique [16]. Lipids were extracted from cells with chloroform: methanol mixture (2: 1, v/v). The sediment was separated by centrifugation (3000 *rpm*, 10 min.) and reextracted twice. The supernatant was mixed with cold water and maintained at 4°C overnight. The lower chloroform phase was used to fractionate the phospholipids by thin-layer chromatography on glass plates HPTLC (Merck) in solvent system chloroform: methanol: ammonium (65: 35: 5). Phospholipid identification was performed using appropriate high purity reagents (Sigma) as markers [17]. Mineralization of lipid phosphorus was carried out in mixture of sulfuric and nitric acids.

Upper water-methanol phase was dialyzed against water (4°C, 72 hrs.). The ganglioside determination was performed by thin-layer chromatography on glass plates HPTLC (Merck) in solvent system chloroform: methanol: ammonium: water (60: 35: 2: 6). Contents of gangliosides was determined with periodat-resorcine method. Identification of ganglyosides was carried out using appropriate markers (Sigma) [17].

The quantity of phospholipids and ganglyosides was evaluated by phosphorus and sialic acid determination in (*g* per 1 *mg* of protein measured by Lowry method [18]. Statistical treatment was performed by Student t-test.

RESULTS. Results of the analysis of phospholipids in bone marrow progenitors, thymocytes and mature lymphocytes derived from intact rats are shown in *Table 1*.

Table 1. Contents of phospholipids (% of the total) in bone marrow progenitors of lymphocytes, thymocytes, and mature lymphocytes in intact animals. (Both thymocytes and mature lymphocytes were compared with bone marrow progenitors. Here and below the mean (standard error are shown, * means $p<0.05$)

Phospholipid classes	Bone marrow progenitors	Thymocytes	Mature lymphocytes
Phosphatidylinositol	6.4 ± 0.5	8.5 ± 0.8	9.6 ± 1.2*
Lysophosphatidylcholine	12.0 ± 0.8	7.7 ± 0.6*	8.8 ± 1.3*
Sphingomyelin	25.6 ± 1.6	21.3 ± 0.9*	15.8 ± 0.9*
Phosphatidylcholine	23.4 ± 1.7	29.9 ± 2.0*	21.9 ± 0.7
Phosphatidylserine	10.4 ± 0.8	9.9 ± 0.7	15.7 ± 0.8*
Phosphatidylethanolamine	12.0 ± 1.1	15.9 ± 1.2*	16.4 ± 1.2*
Cardiolipin	10.4 ± 1.2	6.8 ± 0.7*	12.7 ± 0.8

Expressed modifications of cell phospholipid composition during lymphocyte differentiation were observed. The comparison of phospholipids in bone marrow cells and tymocytes revealed the decrease in lysophosphatidylcholine (LPC), sphingomyelin (SPM), and cardiolipin (CL) and the increase in level of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In mature lymphocytes LPC and SPM levels were lower and phosphatidylinositol (PI), PE and phosphatidylserine (PS) concentrations were higher than in bone marrow progenitors.

The gangliosides of investigated cells (*Table 2*) from intact rats were separated into five fractions: mono-, two fractions of di- (that have different location of neuraminic acid residues, were denoted below as fractions 1 and 2), tri- and tetrasialogangliosides. In thymic lymphocytes fraction of disialogangliosides was more abundant and mono- and tetrasialoganglioside levels were lower than in bone marrow progenitors. In mature lymphocytes the level of all gangliosides except tetrasialogangliosides is the same as in the bone marrow cells. So, alterations in phospholipids and gangliosides during cell maturation seem to be more expressed in thymocytes than in mature lymphocytes.

Table 2. Contents of ganglyosides (% of the total) in bone marrow progenitors of lymphocytes, thymocytes and mature lymphocytes in intact animals. (Both thymocytes and mature lymphocytes were compared with bone marrow progenitors)

Ganglioside classes	Bone marrow progenitors	Thymocytes	Mature lymphocytes
Monosialogangliosides	22.8 ± 1.8	17.6 ± 0.9*	24.6 ± 1.6
Disialogangliosides-1	17.7 ± 1.6	21.3 ± 1.0	18.1 ± 1.1
Disialogangliosides-2	15.8 ± 1.3	22.1 ± 2.0*	17.7 ± 1.3
Trisialogangliosides	19.3 ± 2.0	21.2 ± 1.6	23.8 ± 1.4
Tetrasialogangliosides	24.4 ± 1.9	17.2 ± 0.9*	16.2 ± 0.9*

In cells derived from rats with sarcoma 45 the phospolipid levels (*Fig. 1*) at any stage of lymphocyte maturation showed the drop in PI and PC and the increase in LPC in comparison with normal cells. The increase in SPM was observed in thymocytes and mature lymphocytes. PS elevated in immature cells but decreased in mature ones. PE diminished only in mature cells and CL increased only in thymocytes.

Ganglioside determination in animals with cancer (*Fig. 2*) showed the increase in mono- and disialogangliosides and the decrease in tri- and tetrasialogangliosides in comparison with healthy rats.

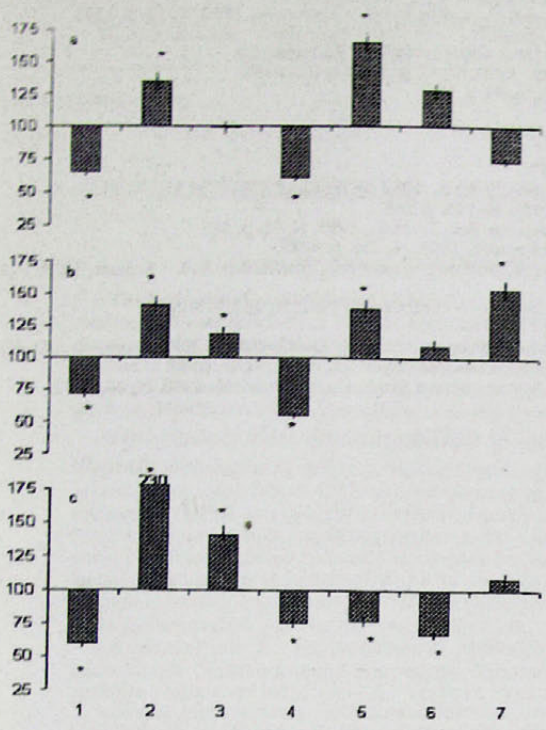

Fig. 1. The contents of phospholipids in tumor-bearing rats (in % of the same in intact animal cells taken for 100%): a – bone marrow progenitor of lymphocyte, b – thymocyte, c – mature lymphocyte. Phospholipid fractions: 1 – phosphatydylinositol, 2 – lysophosphatidylcholin, 3 – sphingomyelin, 4 – phosphatidylcholine, 5 – phosphatidylserine, 6 –phosphatidylethanolamine, 7 – cardiolipin. (Phospholipid fractions of cells from normal and tumor-bearing animals were compared).

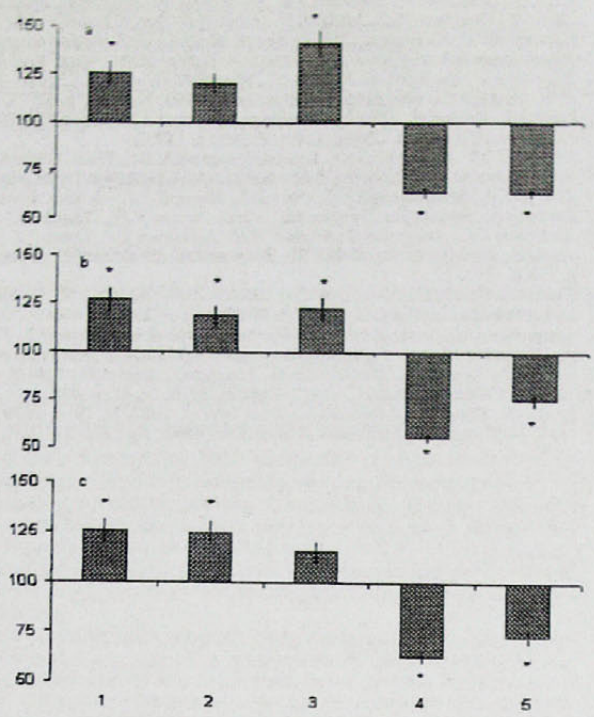

Fig. 2. The contents of ganglioside in tumor-bearing rats (in % of the same in intact animal cells taken for 100%): a – bone marrow progenitor of lymphocyte, b – thymocyte, c – mature lymphocyte. Ganglioside fractions: 1 – monosialoganglioside, 2 – disialoganglioside-1, 3 – disialoganglioside-2, 4 – trisialoganglioside, 5 – tetrasialoganglioside. (Ganglioside fractions of cells from normal and tumor-bearing animals were compared).

DISCUSSION. The results presented demonstrate that phospholipids and gangliosides are actively involved into processes of lymphocyte differentiation and that lipid pattern is susceptible to tumor developing in the organism.

One of the most noticeable alterations in grafted rats is a drop in PI level at all stages of lymphocyte maturation. It is known that PI is involved into intracellular signal transduction as a secondary messenger [19, 20] and that this phospholipid may interact as a ligand with immune cell receptors [21]. So, it may be proposed that described decrease in its level reflects modifications of these functions as a sequence of developing solid tumor influence.

Other expressed shifts, i.e. the decrease in PC level and increase in LPC may suggest the activation of phospholipase A2 mediated hydrolysis of PC with formation of LPC and arachidonic acid [22, 23] in lymphocytes derived from an organism with solid tumor.

As to gangliosides, a reduction in their sialylation level in lymphocytes from the organism with solid tumor was observed. These lipids were demonstrated to play an active role in immune reaction formation, the cell growth and other cell function regulation [24] Gangliosides were proposed to be receptors of lymphocyte membrane [25]. The addition of gangliosedes to a tumor cell inoculum or their systematic administation enhances the tumor formation in mice [26, 27]. Neuroblastoma tumor gangliosides may inhibit lymphoproleferative response to an allogenic stimulus [28].

Our results are a good ground to propose that gangliosides may play an important role in lymphocyte and cancer cell interaction. As sialylation level of gangliosides is connected with their functional activity, the described alteration of this character seems to concern to modification of immune reaction occurring at cancer animals.

Taken together our data suggest alterations of phospholipids and gangliosides occurring during lymphocyte maturation. Dynamics of these shifts is a subject of modification under the influence of tumor developing in the organism.

ABBREVIATION: CL – Cardiolipin; LPC – Lysophosphatidylcholine; PC – Phosphatidylcholine; PE – Phosphatidylethanolamine; PI – Phosphatidylinositol; PS – Phosphatidylserine; SPM – Sphingomyelin.

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