Expression of nicotinic acetylcholine receptors on human B-lymphoma cells

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Aim. *To find a correlation between the level of nicotinic acetylcholine receptor (nAChR) expression and B lymphocyte differentiation or activation state.* ***Methods***. *Expression of nAChRs in the REH, Ramos and Daudi cell lines was studied by flow cytometry using nAChR subunit-specific antibodies; cell prolife- ration was studied by MTT test.* ***Results***. *It is shown that the level of 4 2/ 4 and 7 nAChRs expression increased along with B lymphocyte differentiation (Ramos > REH) and activation (Daudi > > Ramos) and depended on the antigen-specific receptor exp- ression. The nAChR stimulation/blockade did not influence the intensity of cell proliferation.*

Keywords: nicotinic acetylcholine receptor, B- lymphocytes.

Introduction. Nicotinic acetylcholine receptors (nAChRs) mediate fast synaptic transmission in muscles and autonomic ganglia and regulate cognitive processes in the brain [1]. The important role of cholinergic regulation in the processes of cell proliferation, activation, and immune response was determined after the discovery of nAChRs in skin keratinocytes, respiratory epithelium, vascular endothelium [2, 3], lymphocytes and macrophages [4, 5].

Previously we have demonstrated that B-lymphocytes of mice express two subtypes of nAChR, namely, 4(5) 2 and 7, influencing selection/survival of B-lymphocytes in the course of development and their activation in mature state that results in the impact on humoral immune response [6, 7]. 7-containing nAChRs expressed in hybridoma cells regulate their proliferation and production of antibodies [8]. However, it is yet to be investigated whether the mentioned influence may be related to human lymphocytes. These investigations are important for understanding a role of nicotine in the development of tumours of B-lymphocyte origin.

The aim of the current work was to study the expression and pro-proliferative functions of nAChRs in human B-lymphoma cells.

Materials and Methods. REH, Ramos and Daudi cell lines were received from S.P. Sydorenko (R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine). The cells were grown in RPMI 1640 medium with the addition of 20 mM HEPES, 40 μg/ml gentamicin and 10% fetal calf serum (*Sigma*, USA). Rabbit

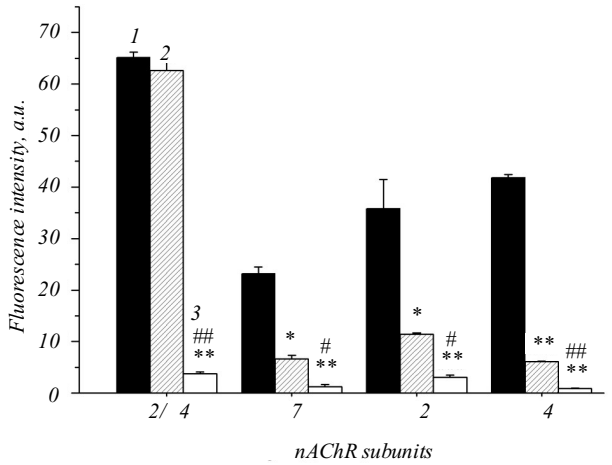

Fig.1 Binding of nAChR-specific antibodies to Daudi (*1*), Ramos (*2*), and REH (*3*) cells in flow cytometry; *p < 0.005; **p < 0.0005 compared to Daudi cells; #p < 0.005; ##p < 0.0005 compared to

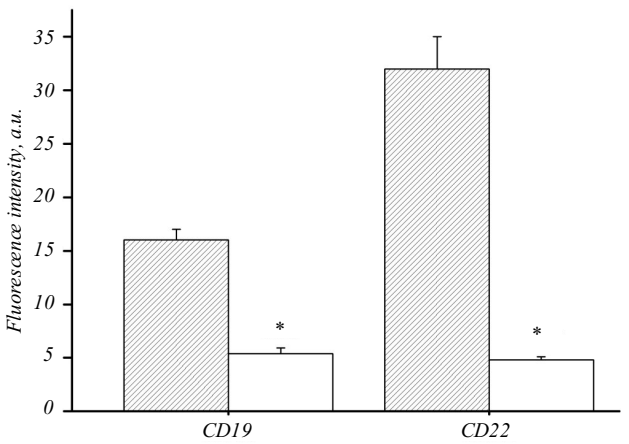

Fig.2 Binding of CD19- and CD20-specific antibodies to Ramos (*1*) and REH (*2*) cells in flow cytometry; *p < 0.0005 compared to

affinitely-purified antibodies against nAChR subunits were generated by us previously [9, 10]. The antibodies against CD19 labelled with fluorescein isothiocyanate (FITC) and the antibodies against CD22 labelled with phycoerythrine (PE) were purchased from *Immunotech* (France), streptavidin labelled with PE – from *Pharmingen* (BD), epibatidine and methyllicaconitine (MLA) – from *Sigma* (USA).

The cells ($1 \cdot 10^6$ in 50 µl of buffered physiological solution with 1% bovine serum albumin) were treated with biotinylated antibodies against 4, 7, 2 or 4 nAChR subunits for 15 min at room temperature. The optimal concentration of antibodies was selected in accordance with the results of immune-enzyme assay with antigenic fragments of subunits. After washing, the cells were incubated with PE-labelled streptavidin for 15 min followed by subsequent washing and analysis with flow cytometer EPICS-XL (*Coulter-Beckman*, France) using corresponding software. Other portions of cells were stained with FITC-labelled antibodies against CD19 and PE-labelled antibodies against CD22 and investigated in a similar way.

The cells seeded in 96-well plates ($1.5 \cdot 10^4$ of cells per well in 100 µl of medium) were incubated with epibatidine (1 and 10 µm) or MLA (25 and 100 nM) at 37°C for 48 hours. The number of living cells was calculated using thiazolyl blue assay [11].

Results and Discussion. The antibodies generated against short fragments of rat nAChR subunits were shown to specifically bind corresponding nAChR subtypes on autonomic ganglia neurons of rats and guinea pigs [9, 10] and on B-lymphocytes of mice [6-8]. The antigenic fragments of 4, 2, and 4 nAChR subunits of both rats and humans are absolutely identical, fragment of 7 subunit contains two conservative substitutions (Lys for Arg and Ser for Asn); therefore, the antibodies should bind to corresponding human nAChRs. The peptide used to generate 4-specific antibody (AVGTYNTRKYEC) is highly homologous to the corresponding fragment of 2 subunits of both rats and humans (ATGTYNSKKYDC), therefore, 4-specific antibody could potentially identify 2-containing nAChRs (UniProtKB (www.uniprot.org)). According to the literature data [4], Daudi cells express mRNA of 2, 5, 6, and 7, but not 4 nAChR subunits; that is why the binding of 4/ 2-specific antibody at least on this cell line was attributed to 2-containing nAChRs.

We compared the binding of nAChR-specific antibodies to lymphoma cells, which correspond to different differentiation stages of B-lymphocytes: pre-B-cells (REH), mature B-lymphocytes (Ramos) and B-lymphoblasts (Daudi) (catalogue numbers ATCC CRL-8286 (REH), CRL-1596 (Ramos), CCL-213 (Daudi) [13, 14]). As shown in Fig. 1, the strongest binding of antibodies was observed in Daudi

cells, and the weakest – in REH cells, i.e. an absolute amount of nAChRs increased with B-lymphocytes maturation and activation. The most significant difference in the binding of 4/ 2-specific antibody was observed for REH and Ramos cells (approximately 16 times in arbitrary units of fluorescence). The binding of 7-, 2-, and 4-specific antibodies in REH compared to Ramos cells was 5-, 4-, and 7-fold higher respectively.

Contrary to REH, Ramos cells express mature form of antigen-specific receptor (BCR) and -chain of its signalling module CD79 (DSMZ online Catalogue http:/www. dsmz.de/human_and_animal_cell_lines). We compared these cells in expression of CD19 (BCR co-receptor) and CD22 (negative regulator of BCR signalling). The data presented in Fig. 2 indicate that Ramos cells express more CD19 and much more CD22 than REH cells. It means that ~~the~~ differentiation of human B-lymphocytes, connected to the expression of antigen-specific receptor, is accompanied with the intensification of 4/ 2-containing nAChRs expression mainly, i.e. this subtype is the most probable regulator of BCR-mediated processes. This finding is in good agreement with the results obtained in mouse B-lymphocytes, where the number of 4-containing nAChRs reaches its maximum in mature B-lymphocytes of the bone marrow after the expression of valid BCR [6].

Ramos and Daudi cell lines, belonging to the same type of tumours (Burkitt's lymphoma), were not identical in the number of nAChRs expressed. Compared to Ramos cells, Daudi cells demonstrated almost similar fluorescence intensity of bound 4/ 2-specific antibody, while the intensity of 7- and 2-specific antibodies was 3-fold, and 4-specific antibodies – 7-fold higher in Daudi cells. Since the stage of Daudi cells differentiation corresponds to activated B-lymphocytes (lymphoblasts) (ATTC: The global bioresource center (www.atcc.org)), the data obtained indicate that B-cell activation is accompanied with the increase of expression of 7-, 2- and 4-containing, but not 4/ 2-containing nAChRs. It is in good agreement with our previous results demonstrating that activation of mouse B-lymphocytes with CD40-specific antibody results in the increase of 7-containing nAChRs expression [12].

Cell proliferation of hybridoma 1D6 (producing antibodies against nAChR 3 subunit) and of chicken pre-B-lymphoma DT40 [13] was blocked by -cobratoxin and MLA, specific antagonists of 7-containing nAChRs [8, 14], i.e. the functioning of this nAChR subtype was required to maintain cell proliferation potential. On the contrary, REH, Ramos, and Daudi cells demonstrated their insensitivity to 7-specific ligands: neither epibatidine (agonist of a wide nAChR spectrum), nor MLA influenced the number of living cells after 3 days of incubation (data not shown).

Taken together, the data obtained demonstrate that similarly to mouse and chicken cells, human cells of B-lymphoid origin contain several nAChR subtypes. The level of their expression increases alongside with the B-lymphocyte differentiation. Different nAChR subtypes are likely to perform non-identical functions, since the increase in the expression of 4/ 2-containing nAChRs accompanies the process of mature antigen-specific receptor (BCR) formation, while the increase in the expression of 7-containing nAChRs goes along with the transition from the stage of mature lymphocyte to lymphoblast. The binding of nAChR-specific ligands (either agonists or antagonists) does not influence the intensity of proliferation of investigated cells.

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Експресія нікотинових ацетилхолінових рецепторів на клітинах В-лімфом людини

Резюме

Мета. *Визначити зв'язок між рівнем експресії нікотинових ацетилхолінових рецепторів (нАХР) і ступенем диференціації та активації В-лімфоцитів.* ***Методи***. *Експресію нАХР на клітинних лініях REH, Ramos і Daudi вивчали методом протокової цитофлуориметрії з використанням антитіл, специфічних до різних субодиниць рецептора; проліферацію клітин оцінювали за допомогою МТТ-тесту.* ***Результати***. *Показано, що рівень експресії 4 2/ 4 та 7 нАХР підвищується по мірі ди- ференціації (Ramos > REH) і активації В-лімфоцитів (Daudi > Ramos) та залежить від експресії антиген-специфічного рецептора. Стимуляція/блокування нАХР не впливає на інтенсивність проліферації досліджених клітин.*

Ключові слова: нікотиновий ацетилхоліновий рецептор, В-лімфоцити.

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Экспрессия никотинових ацетилхолинових рецепторов на клетках В-лимфом человека

Резюме

***Цель**. Определить связь между уровнем экспрессии никотинових ацетилхолинових рецепторов (нАХР) и степенью дифференциации и активации В-лимфоцитов. **Методы**. Экспрессию нАХР на клеточных линиях REH, Ramos и Daudi изучали методом проточной цитофлуориметрии с использованием антител, специфичных к разным субъединицам рецептора; пролиферацию клеток оценивали по результатам МТТ-теста. **Результаты**. Показано, що уровень экспрессии 4 2/ 4 и 7 нАХР повышается по мере дифференциации (Ramos > REH) и активации В-лимфоцитов (Daudi > Ramos) и зависит от экспрессии антиген-специфического рецептора. Стимуляция/блокирование нАХР не влияет на интенсивность пролиферации исследованных клеток.*

Ключевые слова: никотиновий ацетилхолиновий рецептор, В-лимфоциты.

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