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PNA - and SBA-positive lymphocytes content in the major salivary glands' structures during early postnatal period after intrauterine antigenic action

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Key words: Salivary Glands, Peanut Agglutinin, Soybean Agglutinin, Lymphocytes, Rats.

Aim – to determine the Peanut agglutinin- and Soybean agglutinin-positive (PNA+, SBA+) lymphocytes content in the major salivary glands' structures in early postnatal period after intrauterine antigenic action.

Methods and results. 224 submandibular salivary glands of white laboratory rats were investigated through the lectin histochemical and statistical methods.

Conclusions. In newborn animals after intrauterine antigen action the increased content of PNA+ intraepithelial lymphocytes and PNA+ stromal lymphocytes of major salivary glands was determined as compared to control group. That tendency remains till the 14th day, gradually decreasing till the 45th day of postnatal formation. Quantity of SBA+ lymphocytes decreased in antigen premium animals as compared with control group animals. In addition, the quantity of SBA+ lymphocytes in experimental group remains lower during two weeks after birth. On the 45th day of postnatal formation the findings do not differ among experimental animals.

Вміст PNA- та SBA-позитивних лімфоцитів у структурах великих слинних залоз щурів у ранньому постнатальному періоді після внутрішньоутробної антигенної дії

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З метою визначення вмісту лімфоцитів із рецепторами до лектину арахісу та лектину сої (PNA+, SBA+) у структурах великих слинних залоз щурів у ранньому постнатальному періоді після внутрішньоутробної антигенної дії вивчили 224 підщелепні слинні залози білих лабораторних щурів із використанням лектингістохімічного та статистичного методів дослідження. У новонароджених тварин після внутрішньоутробної дії антигену встановили більший вміст PNA+ рецепторів на плазматичній мембрані інтраепітеліальних лімфоцитів і лімфоцитів стромы великих слинних залоз у порівнянні з контрольною групою. Вміст PNA+ лімфоцитів у паренхимі та стромі слинних залоз поступово знижується до 14 доби післянатального розвитку. Знижений вміст SBA+ лімфоцитів в експериментальній групі щодо інтактної зберігається протягом перших двох тижнів після народження з поступовим зменшенням їхньої кількості до завершення експерименту.

Ключові слова: слинні залози, лектин арахісу, лектин сої, лімфоцити, щури.

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Содержание PNA- и SBA-положительных лимфоцитов в структурах больших слюнных желез крыс в раннем постнатальном периоде после внутриутробного антигенного действия

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С целью определения содержания лимфоцитов с рецепторами к лектинам арахиса и сои (PNA+, SBA+) в структурах больших слюнных желез крыс в раннем постнатальном периоде после внутриутробного антигенного действия изучено 224 подчелюстных слюнных железы белых лабораторных крыс с использованием лектингистохимического и статистического методов исследования. У новорожденных животных после внутриутробного действия антигена установлено большее содержание PNA+ рецепторов на плазматической мембране интраэпителиальных лимфоцитов и лимфоцитов стромы больших слюнных желез в сравнении с контрольной группой. Содержание PNA+ лимфоцитов в паренхиме и строме слюнных желез постепенно уменьшается до 14 суток постнатального развития. Пониженное содержание SBA+ лимфоцитов в экспериментальной группе относительно интактной сохраняется в течение первых двух недель после рождения с постепенным уменьшением их количества к окончанию эксперимента.

Ключевые слова: слюнные железы, лектин арахиса, лектин сои, лимфоциты, крысы.

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Background

Salivary glands' diseases have a varied etiology that includes autoimmune, infectious, idiopathic, and hereditary causes. The unifying factor in these diseases is the formation of granulomas, which histologically are mononuclear inflammatory cells or macrophages surrounded by lymphocytes [5]. It is also known that salivary glands NK cells, although related to conventional NK cells, are a distinct subset of innate lymphoid cells that deviates from the conventional developmental pathway, perhaps under the influence of tissue-specific factors [4]. Improved the lymphocytes

population influence on a formation of different organs and systems. Immature lymphocytes migrated from thymus to the peripheral organs of immune system [9]. One of the determinatives that result in violation of major salivary glands morphogenesis and as a result the development of its pathology is the condition of pregnant women health, more than half of them have chronic diseases and system functional disorders which are accompanied by the immune pathological condition, namely by antigen influence on a fetus [3,9]. In the literature there are no systematic data concerning the intrauterine antigen influence on morphogenesis of major

salivary glands and lymphoid tissue associated with it. Using the lectin histochemistry methods appeared a possibility to observe the immature and γ/δ – lymphocytes populations in the major salivary glands' structures after intrauterine antigen action.

Objective

To determine the PNA+ and SBA+ lymphocytes content in the major salivary glands' structures in early postnatal period after intrauterine antigen action.

Methods

The objects of the research were 224 salivary glands of white laboratory rats. Due to impossible qualitative materials taking in the early periods of postnatal life from parotid and sublingual salivary glands the investigation was carried out on the submandibular salivary glands. The rats were divided into three groups. First group are intact rats. Second group are rats, to which 0,05 ml solution of antigen were injected into the amniotic fluid on the 18th day of pregnancy by the method of N. Voloshyn [10], the third group – control, 0,05 ml of physiological solution were injected to the animals intrauterinely on the 18th day of pregnancy. The feeding of animals was twice a day at the same time. For the study of peculiarities of PNA+ and SBA+ lymphocytes content in the major salivary glands structures after antigen's action on the fetus, the model of transuterine, transmembrane injection of antigen into amniotic fluid by the method of N. Voloshyn was chosen [10]. The antigen was liquid (killed) split – vaccine Vaxigrip 2009. Keeping the animals and experiments were carried out accordingly to regulations of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.86), law of Ukraine «About protection of animals from cruel treatment» (№ 1759 from 15.09.2009). The animals killing and taking of the material were done from 13-00 till 14-00 on the 1st, 5th, 7th, 11th, 14th, 30th, 45th days of postnatal life. On every term in all groups of the animals were examined 5–6 animals from 2–3 afterbirths. For the investigation the major salivary glands were used during some minutes after killing. The samples were fixed in 10% solution of formalin, dehydrated, filled in paraffin mixture and 4 μm thick serial paraffin sections were produced. The sugar residues determination of β -D-galactose and N-acetyl-D-galactosamine was investigated using the standard method [2] through peanut agglutinin (PNA) and soybean agglutinin (SBA) using the standard kit of NPA «Lectin Test», Lviv. For visualization 3,3'-diaminobenzidine was used as the staining agent. The results of account of lectin histochemistry were done semi-quantitatively and determined as +++ - dark brown stain, ++ - brown stain, + - pale brown stain, zero is absence of stain. Intermediate tones were evaluated accordingly: ++/+++ , +/+++ . PNA+ receptors distribution was detected on the intraepithelial lymphocyte plasmatic membrane and stromal lymphocytes of major salivary glands. SBA+ receptors were detected on the stromal lymphocyte plasmatic membrane [5]. The PNA+ and SBA+ lymphocytes quantities were counted per notional area unit through A. Glagolev's modified ocular grid with the findings recalculation per 10000 μm^2 (1000 magnification). Process-

ing of the obtained numerical results was conducted through statistical methods using STATISTICA® for Windows 6.1 (StatSoft Inc., № AXXR712D833214FAN5). Comparison of variables was performed using Student's t-test.

To verify existence of relationship between obtained variables correlation analysis (Pearson correlation coefficient) was used. The difference between the variables was considered statistically significant at the $p \leq 0,05$.

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Results

In the acini, PNA+ lymphocytes are situated among the serous and mucous secretory cells, often near the cells with mitosis figures. Intraepithelial lymphocytes are present mainly as the small forms. There are the round cells with a peripherally pigment deposition and pericytoplasmic enlightenment.

On the first day after birth the PNA+ lymphocytes quantity in the intact group is $19,8 \pm 1,13$. In antigenpremium rats the intraepithelial PNA+ lymphocytes content is increased compared with intact group – $24,3 \pm 1,32$. Indicators of all above-stated structures for the animals of control group do not differ from the data obtained from the animals of intact group, that is why in the future control group will not be cited (fig. 1).

On the 5th day of postnatal life, the quantity of intraepithelial lymphocytes with PNA receptors decreases in intact animals group compared with newborn rats. In experimental group the PNA+ lymphocytes content is significantly more than in intact group – $22,3 \pm 1,61$ ($p \leq 0,05$). On the 7th day, in intact animals group PNA+ lymphocytes quantity decrease relative to the previous observation term is observed. In experimental group the tendency of PNA + lymphocytes content increase is saved compared with intact animals. On the 11th day of postnatal life in observed animals from 1st group the quantity of lymphocytes with β -D-galactose receptors is continuing to decrease gradually compared with previously observed term. In experimental group the PNA + lymphocytes content is remaining greater relative to the intact group (fig. 1). On the 14th day after birth in intact animals group the intraepithelial PNA+ lymphocytes quantity remains at the level of previously observed term but in experimental group the PNA+ lymphocytes content is statistically significantly more compared with control animals.

The intraepithelial lymphocytes quantity on the 30th day of postnatal life in intact group is somewhat reduced compared with the data from animals of 14th day after birth. In experimental animals group the content of lymphocytes with PNA+ receptors is decreasing to the indicators in intact group of the current observation period. At the 45th day of postnatal life, the PNA+ lymphocytes quantity remains at the level of previously observed term. Statistically significant is no difference between the indicators of investigated parameters (fig. 1).

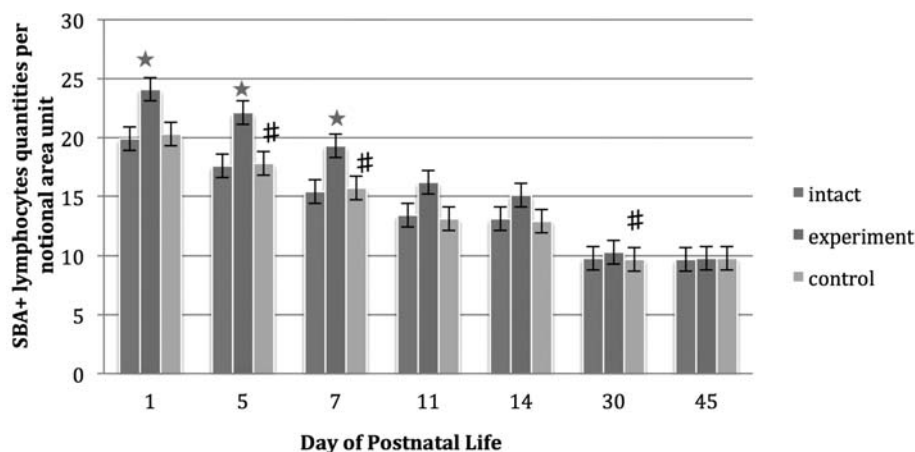


Fig. 1. PNA+ lymphocytes content in major salivary glands' parenchymal structures per notional area unit ($10000 \mu\text{m}^2$).

Notes: ★ – result is significant compared with intact group; # – result is significant compared with previously observed term.

PNA+ lymphocytes are situated among the fibroblasts of major salivary glands' stromal structures. On the 1st day after birth in intact animals group the quantity of lymphocytes with PNA receptors is total $2,2 \pm 0,09$ per $5000 \mu\text{m}^2$ of the stromal structures. In experimental animals the PNA+ lymphocytes content was significantly more than in intact group and was $3,1 \pm 0,22$ per $5000 \mu\text{m}^2$. The intact group indicators significantly do not differ from control group results (fig. 2).

On the 5th day of postnatal life in the control group the PNA+ lymphocytes quantities in the stromal structures increase relative to previously observed term.

In experimental group the quantity of the small lymphocytes with β -D-galactose residues remains statistically significantly more ($3,8 \pm 0,11$) as compared with indicators of intact group ($2,8 \pm 0,13$). On the 7th day of postnatal formation the quantity of lymphocytes with PNA receptors in all observed groups is the maximum during experiment. In antigenpremium animals their content remains more ($4,1 \pm 0,1$) relative to the control group ($3,4 \pm 0,1$; $p < 0,05$). On the 11th day in intact group among the stromal cells the PNA+ lymphocytes quantity decrease is observed and their content is $2,3 \pm 0,1$ per notional area unit. In the experimental group their quantity remains more than the results of control

group and is $2,6 \pm 0,08$ per notional area unit. At the 14th day of postnatal life in the stromal structures of intact animals the PNA+ lymphocytes quantities decrease compared with previously observed term. In the experimental group their part remains more than indicators of control group $2,0 \pm 0,09$. The PNA+ lymphocytes quantities decrease tendency is observed till the end of the experiment (fig. 2). On the 30th day the PNA+ lymphocytes content in experimental group is decreased to $1,4 \pm 0,1$. The results obtained in the experimental group do not significantly differ from the indicators in the control group. On the 45th day of postnatal life a tendency to decrease of quantities of lymphocytes with PNA receptors in the rats' salivary glands' stromal structures remains in all investigated groups. The data statistically does not differ in all observed groups (fig.2).

The SBA+ lymphocytes are located diffusely in the capsule, septa and ducts of the major salivary glands. In newborns animals of intact group the quantity of SBA+ lymphocytes with phenotypic features of small forms and intermediate forms is $1,9 \pm 0,1$ per notional area unit. In experimental animals of the 1st day after birth the lymphocytes with SBA+ receptors quantity decrease is observed compared with intact animals group. In the control group the SBA+

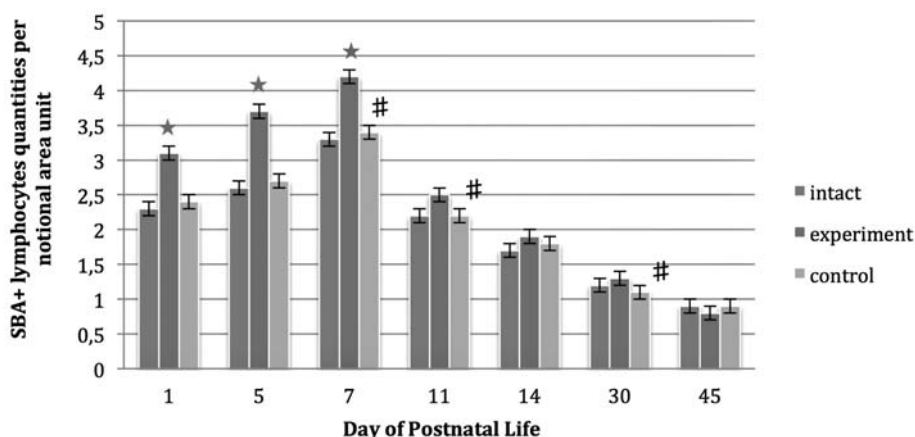


Fig. 2. PNA+ lymphocytes content in major salivary glands' stromal structures per notional area unit ($10000 \mu\text{m}^2$) according to the age and observation group.

Notes: ★ – result is significant compared with intact group; # – result is significant compared with previously observed term.

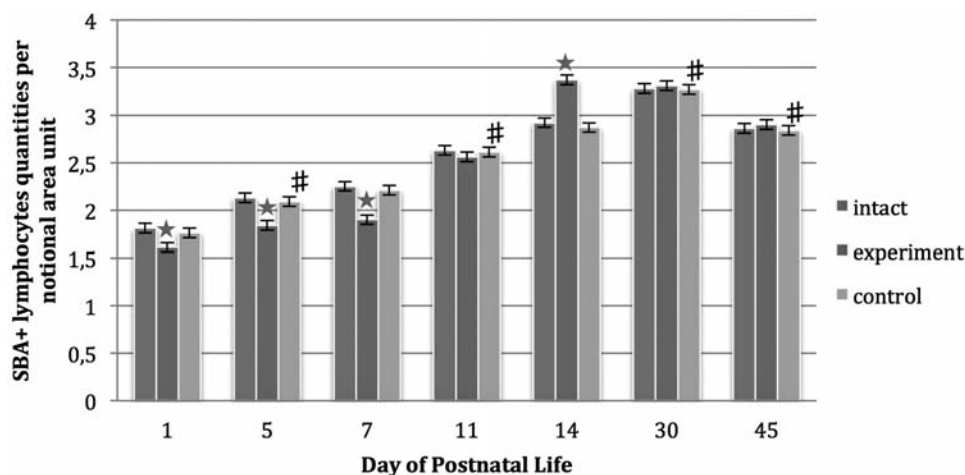


Fig. 3. SBA+ lymphocytes content in major salivary glands' stromal structures per notional area unit (10000 μm^2).

Notes: ★ – result is significant compared with intact group; # – result is significant compared with previous observation term.

lymphocytes quantity does not differ significantly from the indicators of intact group from the neonatal period till the end of experiment. On the 5th day in the intact group the tendency to increase of number of SBA+ lymphocytes is detected relative to the newborn animals (fig. 3). In the experimental group the quantity of lymphocytes with N-acetyl-D-galactosamine residues on the receptors is $1,8 \pm 0,09$ that is more than in newborn rats and significantly less relative to the intact group.

On the 7th day the tendency to quantity increase of SBA+ cells with phenotypic features of small and intermediate lymphocytes forms in intact group remains relative to previously observed term. For experimental animals the indicators of SBA+ lymphocytes quantity remain lower as compared with the intact animals (fig. 3). The 11th day of postnatal life is characterized by increased quantity of lymphocytes with SBA receptors in intact group. The SBA+ lymphocytes content in the major salivary glands' connective tissue relative to the cells number is $2,7 \pm 0,1$. For experimental animals the total amount of SBA+ lymphocytes is lower than in the control group. On the 14th day, in the major salivary glands' connective tissue of intact animals group the quantity of SBA+ cells with phenotypic features of the small and intermediate lymphocytes is increased as compared with previously observed term (fig. 3). At first, unlike the previously observed period, in experimental group the SBA+ lymphocytes quantity is $3,5 \pm 0,1$, that is significantly more than the results of intact animals. On the 30th day after birth, the SBA+ lymphocytes quantity, as compared with indicators of the 14th day, increases by 0,38 per notional area unit. Indicators of the lymphocytes with SBA+ receptors obtained from experimental group are at the level of the control group indicators (fig. 3). On the 45th day of postnatal formation, the quantity of SBA+ cells with phenotypic features of the small and intermediate lymphocytes in the major salivary glands' connective tissue is significantly decreased relative to the previous observation term. The results obtained in experimental and intact groups during this observation period do not differ.

Discussion

Salivary gland mononuclear cells are composed of a variety of subpopulations distributions of which differ between parotid (PG) and submandibular salivary glands (SMG) and are distinct from lymphatic node, both PG and SMG were enriched in immature and activated T cells. Unchanged percentages of Thy-1(+) T cells in PG and SMG following short-term adult thymectomy indicated that immature salivary gland T cells had an extrathymic source [6]. The SMG considered a privileged site where dissemination of the virus to other tissues as well as transmission to native individuals is possible. The SMG provides a peculiar barrier where mucosal tissues are typically poised to respond in a Th2 fashion and stimulate the production of IgA, while denying access to commensal microbes and other environmental pathogens [8]. Sugar residues are important components of salivary gland secretion. Acinar cells contained abundant quantities of glycoconjugates with the terminal trisaccharide sialic acid – (alpha 2–3, 6) galactosyl (beta 1–3) N-acetylgalactosamine. Mandibular acinar cells also contained alpha and beta N-acetylgalactosamine and N-acetylglucosamine residues, whereas the demilunar cells contained glycoconjugates with fucose, mannose and N-acetylglucosamine residues. In the duct system a range of sugar residues was localized throughout the cell cytoplasm or limited to the apical surface [7]. Secretions in parotid and submandibular serous cells generally contained a higher content of fucose than those in sublingual serous cells, which contained higher levels of a terminal galactose-sialic acid dimer. Some but not other cells of striated and interlobular ducts of submandibular glands of one subject were stained for alpha-N-acetylgalactosamine [1].

Our study showed that in group of animals after antigen action in fetal period the PNA+ lymphocytes quantity increase is observed. Among them there are two populations – immature forms and γ/δ lymphocytes [9]. Thus, the PNA+ lymphocytes quantities dynamics have an undulatory character. In newborn animals group after intrauterine antigen action till the 7th day of postnatal life the PNA+ lymphocytes number

increase is noted as compared with the control group. From the 11th day of postnatal life and till the end of experiment the PNA+ lymphocytes quantities are gradually decreased and on the 45th day the difference between the experimental and intact group indicators are not determined. Furthermore, from the neonatal period till the 11th day content of SBA+ cells with phenotypic features of the small and intermediate lymphocytes in the major salivary glands' connective tissue in experimental animals group is lower than results obtained from the intact group. From the 14th day after birth the tendency to quantity increasing is observed, it is saved during the first month of postnatal life and levelled on the 45th day. The similar dynamic of PNA+ and SBA+ lymphocytes distribution in the epithelium and connective tissue of throat, gums and periodontium is described by several authors earlier and confirms the lymphocytes role in organs' morphogenesis [3].

Conclusions

1. In newborn animals after intrauterine antigen action the increased content of PNA+ intraepithelial lymphocytes and PNA+ stromal lymphocytes of major salivary glands is determined as compared with control group. The lymphocytes content gradually decreases in glands parenchyma and in the stromal structures till the 14th day of postnatal formation.

2. Quantity of SBA+ lymphocytes is decreased in antigen-premium animals as compared with control group animals. In addition the quantity of SBA+ lymphocytes in experimental group remains lower during two weeks after birth. On the 45th day of postnatal formation the findings do not differ among experimental animals.

Further researches prospects

In our further researches we will investigate distribution of different carbohydrates to define state of the microenvironments of the major salivary glands' structures.

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