Yu. Burega

Zaporizhzhya State Medical University

DOI: https://doi.org/10.26641/1997-9665.2015.3.8-11
UDC: 611.311-018.53:[547.963:634.58].08+616.311.2-018.53-053.13-097.1-092.9]:599.323.4

DISTRIBUTION FEATURES OF LYMPHOCYTES WITH PEANUT AGGLUTININ POSITIVE RECEPTORS IN GUMS EPITHELIUM OF RATS IN NORM AND AFTER INTRAUTERINE ANTIGENIC ACTION

Key words: lymphocytes, peanut agglutinin, gingival mucous epithelium, rat.

The work is the fragment of SRW «Lectinhistochemical characteristics of morphogenesis of the organs and tissues in early postnatal period in norm and experiment» (Number of state Registration 0109U003986).

ABSTRACT. Background. According to the conception "Lymphocyte is the main factor of morphogenesis" changes in lymphocyte receptor repertory, induced by antigenic action in the fetal period of development, influence on organs and tissues development after birth. Functional activity of immunological immature PNA+ lymphocytes inducing the change in functioning, imbalance in formation cells of microenvironment, synthesis of intracellular substance and the fibers of extracellular matrix leads to violation of morphological and functional condition of organs. **Objective.** Determine the features of distribution of lymphocytes with receptors to peanut agglutinin in gingival epithelium of rats in norm and after intrauterine antigenic action. **Methods.** The object of the research was: 224 jaws of 112 white laboratory rats. The rats divided into three groups. First group – intact rats. Second group – rats, which were introduced 0,05 ml solution of antigen in the amniotic fluid on the 18th day of pregnancy by the method of N. Voloshyn, the third group – control, the animals were introduced intrauterine 0,05 ml of physiological solution on the 18th day of pregnancy. The antigen was split vaccine Vaxigrip 2009. **Results and conclusion.** In newborn animals, after intrauterine antigen action it was determined significantly increased content of PNA+ lymphocytes in the epithelium of gingival mucous, compared with control group, where PNA+ lymphocytes number gradually decreases. On the 11th day of life, in animals of second group, quantity of intraepithelial PNA+ lymphocytes remains higher. On 45th day of postnatal formation its share does not significantly differ from similar indicators in all groups and decreases compared with neonatal period.

Morphologia. – 2015. – Т. 9, № 3. – С. 8-11.

✉ axios.ua@gmail.com

Надійшла: 13.08.2015
Прийнята: 10.09.2015

Бурега Ю.О. Особливості розподілу лімфоцитів з рецепторами до лектину арахісу в епітелії ясен щурів в нормі та після внутрішньоутробної антигенної дії.
Реферат. У роботі встановлена динаміка розподілу PNA+ лімфоцитів в слизовій ясен щурів в нормі та після внутрішньоутробної впливу антигену. На першу добу після народження, серед клітин базального шару епітелію ясен у антигенпремійованих в плідному періоді тварин спостерігається максимальний вміст PNA+ лімфоцитів, який є достовірно більшим в порівнянні з показниками тварин інтактної групи. Протягом експерименту, у всіх групах тварин кількість PNA+ лімфоцитів в епітелії ясен поступово зменшувалась, в порівнянні з попередніми термінами спостереження, проте до 11-ї доби життя вміст PNA+ лімфоцитів у антигенпремійованих тварин, залишався достовірно більшим, відносно групи контролю. На 45-ту добу їх частка достовірно не відрізнялася від аналогічних показників всіх груп спостереження та зменшувалася, в порівнянні з періодом новонародженості майже втричі.
Ключові слова: лімфоцит, лектин арахісу, епітелій яснен, щури.

Citation:
Burega Yu. Distribution features of lymphocytes with peanut agglutinin positive receptors in gums epithelium of rats in norm and after intrauterine antigenic action. Morphologia. 2015;9(3):8-11.

Background

Periodontal diseases usually known as gingivitis and periodontitis are widespread in different age categories of population [1]. The innate immune response is a very important compound of immune reaction and constitutes a homeostatic system, which is the first line of defense. Interestingly, the T cell-mediated adaptive immunity development is highly dependent on innate immunity-associated antigen presenting cells, which after antigen capture undergo into a maturation process and migrate towards the lymph nodes, where they produce distinct patterns of cytokines that will contribute to the subsequent polarization and activation of specific T CD4+ lymphocytes [2]. One of the determinatives that result in violation of oral mucous morphogenesis, in particular gingival epithelium, and as a result the development of its pathology is the condition of pregnant health, more than half of them has chronic diseases and system functional disorders which is accompanied by the immune pathological condition, namely by antigen influence on a fetus [3]. According to conception "Lymphocytes - the main factor of morphogenesis" [4] the change of lymphocytes' receptor repertory, that inducing by antigenic action in the fetal period of formation, influence to the organs and tissues development of organism after birth. Disorders in the proliferation, differentiation and migration processes' of lymphocytes after intrauterine injection of various nature antigens, leads to population of immunological immature PNA+ lymphocytes in peripheral lymphoid organs [5, 6]. Functional activity of immunological immature PNA+ lymphocytes inducing the change in functioning, imbalance in formation cells of microenvironment, synthesis of intracellular substance and the fibers of extracellular matrix, that leads to violation of organs' morphofunctional condition, generally [6]. In the literature there are no systematic data concerning the intrauterine antigen influence to morphogenesis of gingival epithelium in early postnatal period. Using the peanut agglutinin for methods of lectinhistochemical detection of β-D-galactose residues appeared a possibility to observe the immature and γ/δ – lymphocytes populations in the gingival epithelium after intrauterine antigen action.

Objective

Determine the features of distribution of lymphocytes with receptors to peanut agglutinin in gingival epithelium of rats in norm and after intrauterine antigenic action.

Methods

The object of the research was: 224 jaws of 112 white laboratory rats. The rats divided into three groups. First group is intact rats. Second group is rats, which were introduced 0,05 ml solution of antigen in the amniotic fluid on the 18th day of pregnancy by the method of N. Voloshyn [1], the third group – control, the animals were introduced intrauterine 0,05 ml of physiological solution on the 18th day of pregnancy. For the study of peculiarities of PNA+ lymphocytes content in the gingival epithelium after antigen's action on the fetus, chosen the model of transuterine, transmembrane introduction of antigen in amniotic waters by the method of N. Voloshyn [1]. The antigen was rare (killed) split - vaccine Vaxigrip 2009. The animals' killing and taking of the material done from 13-00 till 14-00 on the 1st, 5th, 7th, 11th, 14th, 30th, 45th day of postnatal life. Keeping the animals and experiments were carried out accordingly to regulations of European convention about the defense of spine animals', which are used due to the experimental and other scientific aims (Strasbourg, 18.03.86), low of Ukraine "Animal Protection from Cruel Appeal" (№ 1759 from 15.09.2009). On every term in all groups of the animals were examined six animals from 2 - 3 afterbirth. For the investigation, the jaw specimens with the mucous in the area of the molars used during some minutes after killing. The samples fixed in 10% solution of formalin, dehydrated, filled in paraffin mixture and produced serial paraffin sections 4 μm thick. Determination of β-D-galactose sugar residues was investigated using the standard method [5] through peanut agglutinin (PNA) using the standard kit of NPVK "LectinTest", Lviv. For visualization was used 3.3'- diaminobenzidine – hydrogen peroxide system as the staining agent [5]. The count of PNA+ lymphocytes quantity in gingival epithelium done per 1000 epitheliocytes, thus hereinafter in the text the indicators of PNA+ lymphocytes quantity will designate in promille (‰) (1000 magnifications). Processing of the obtained numerical results conducted through statistical methods - STATISTICA® for Windows 6.1 (StatSoft Inc., № AXXR712D833214FAN5). Comparison of variables performed using Student's test. To verify existence of relationship between obtained variables used correlation analysis (Pearson coefficient of correlation). The difference between the variables was considered statistically significant at the $p \leq 0,05$.

Results and discussion

In multilayer keratinizing gingival' epithelium the PNA+ lymphocytes are determining among epitheliocytes of basal and prickle layers. Intraepithelial lymphocytes in the gingiva present mainly the small forms. There are the round cells with a peripherally pigment deposition and pericytoplasmic enlightenment.

On the first day after birth among the basal layer cells of gingival' epithelium in animals of intact group the PNA+ lymphocytes quantity counting 19,9±1,16‰. In antigenpremium animals the PNA+ lymphocytes content in gingival mucous is more than in animals of intact group and is 24,1±1,35‰ ($p \leq 0,05$).

Indicators of control group does not significant differ from the results of intact group, thus hereinafter in the text its will not cite (Tab.1). The terms "intact" and "control" groups are using as syn-

onyms.

On the 5th day of life the quantity of intraepithelial lymphocytes, that have receptors to peanut agglutinin decreased in animals of intact group, compared newborn rats (Tab.1). In animals of antigenpremium group the PNA+ lymphocytes content among the epitheliocytes of basal layer is significant higher, compared intact animals and is 22,1±1,67 ‰ ($p \leq 0,05$).

In intact animals on the 7th day of postnatal life observed the decrease quantity of intraepithelial PNA+ lymphocytes relatively previous observation term. In animals, that were expose by antigenic action in the antenatal period, saving the tendency of increasing content of lymphocytes affinity to peanut agglutinin, compared group of intact animals (Tab.1).

On the 11th day of life in experimental animals of the first group, compared previous observation term, the quantity of lymphocytes with β-D-galactose residues receptors continues gradually decrease. In experimental rats of the second group the PNA+ lymphocytes content remains more, relatively intact group (Tab.1).

Table 1

Quantity of intraepithelial PNA+ lymphocytes in gingival mucous relatively epitheliocytes quantity (‰)

Animals groups	Day of life						
	1	5	7	11	14	30	45
I	19,9±1,1	17,6±1,1	15,4±1,4	13,4±0,9	13,1±0,6	9,8±0,4	9,7±08
II	24,1±1,1*	22,1±1,6*	19,3±1,1*	16,2±1,1*	15,1±0,1	10,3±0,9	9,8±0,7
III	20,3±1,1	17,8±1,1	15,7±1,3	13,1±0,6	12,9±0,3	9,7±0,8	9,8±01

Notes: I – Group of intact animals; II - Group of experimental animals; III – Group of control animals; * - result is significant compared intact group.

On the 14th day of postnatal life in animals of intact group the quantity of intraepithelial PNA+ lymphocytes in the basal layer of gingival epithelium remains at the level of previous observation term, thought in antigenpremium group the PNA+ lymphocytes content statistically significant higher, compared control.

The lymphocytes quantity on the 30rd day of postnatal life in intact group slightly decreases, compared results in animals on the 14th day. In experimental animals of the second group, the content of lymphocytes with receptors to peanut agglutinin decreasing to the indicators of intact animals on the current observation term.

On the 45th day of life the PNA+ lymphocytes quantity in the basal layer of gingival epithelium remains at the level of previous observation term. Statistically significant difference between indicators of the observation parameters was not determined (Tab.1).

Accordingly to conception “Lymphocyte – the factor of morphogenesis” [4] the antigen effect influence in the antenatal period leads to migration of immature lymphocytes, that did not die, from fetus’ thymus to the lymphoid and non-lymphoid organs [4, 6]. By the researches was determined the dynamics of PNA+ lymphocytes in the gingival mucous in norm and after intrauterine antigenic action. On the first day after birth, among the cells of gingival epithelium basal layer in antigenpremium in fetal period animals, observed maximal content of PNA+ lymphocytes in gingival mucous, that is significantly higher, compared animals of intact group. During the experiment in all animals group the quantity PNA+ lymphocytes quantity in gingival epithelium gradually decrease in comparison with previous observation terms. The difference in quantity of lymphocytes with β-D-galactose residues saving still 14th day of life. From the 30rd day determined a reduction of its quantity. On the 45th day its part significantly does not differ from similar indicators of all groups and decrease in comparison with the neonatal period almost three times. The similar dynamic of distribution PNA+ lymphocytes in the epithelium of different morphological structures described by several authors early [7, 8] and confirms the lymphocytes role in organ’s morphogenesis.

Conclusion

In newborn animals, after intrauterine antigen action determined the significant increased content of PNA+ lymphocytes in the epithelium of gingival mucous, compared control group, which gradually decreases. On 11th day of life, in antigenpremium animals, quantity of intraepithelial PNA+ lymphocytes remains higher. On 45th day of postnatal formation its share did not significantly differ from similar indicators in all groups and decreases compared neonatal period, almost three times.

Research perspectives

In our further researches, we will study the distribution features of different sugar residues define state of the microenvironments of rat’s gingival mucous epithelial cells after intrauterine antigenic action.

References

1. Voloshyn MA, Matvieishyna TM, Hrinivetska NV, Bureha YuO, Talanova OS, inventors; Zaporizhia State Medical University, Voloshyn MA, Matvieishyna TM, Hrinivetska NV, Bureha YuO, Talanova OS, assignee. Simulation method for antigen antenatal action. Ukraine patent UA 63020. 2011 Feb 25. Int. Cl. G09B 23/28. Ukrainian.

2. World Health Organization. Design of an oral health survey. In: Oral health surveys basic methods. 5th ed. France; 2013. 13-21.

3. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernández M, Gamonal J. Host response mechanisms in periodontal diseases. J Appl Oral Sci. 2015 May-Jun;23(3):329-55. doi: 10.1590/1678-775720140259. PMID: 26221929.

4. Voloshyn NA. [Lymphocyte – the factor of morphogenesis]. Zaporozhye medical journal. 2005;5:122. Russian.

5. Lutsik AD, DetyukYeS, Lutsik MD authors; Panasyuk YeN editor: [Lectins in histochemictry]. Lviv: Vyshcha shkola; 1989. 144. Russian.

6. Voloshyn NA, Grygoryeva EA. [Experimental model of undifferentiated connective tissue dysplasia syndrome]. Pathologia. 2009;6(1):39-42. Russian.

7. Maslova IN. PNA- and SBA-positive lymphocytes content in the major salivary glands' structures during early postnatal period after intrauterine antigenic action. Pathologia. 2014;(3):78-82.

8. Voloshin N, Svetlitsky A. [Features of PNA+- and SBA+-lymphocytes dynamics in structures of animals small and large intestine in early postatal period of development after antigen introdacton] Ukrainskyi morfologichnyi almanakh. 2008;6(1):153-4. Russian.

Бурега Ю.А. Особенности распределения лимфоцитов с рецепторами к лектину арахиса в эпителии десен крыс в норме и после внутриутробного действия антигена.

Реферат. В работе установлена динамика распределения PNA+ лимфоцитов в слизистой десен в норме и после внутриутробного антигенного действия. В первые сутки после рождения среди клеток базального слоя эпителия десны у антигенпремированных в плодном периоде животных наблюдается максимальное количество PNA+ лимфоцитов, достоверно большее, в сравнении с животными интактной группы. В течение эксперимента во всех группах животных количество PNA+ лимфоцитов в эпителии десен постепенно уменьшалось по сравнению с предыдущими сроками наблюдения, но до 11-х суток жизни количество PNA+ лимфоцитов у антигенпремированных животных оставалось достоверно большим относительно группы контроля. На 45-е сутки, их доля достоверно не отличается от аналогичных показателей всех групп наблюдения, и уменьшается по сравнению с периодом новорожденности почти в три раза.

Ключевые слова: лимфоцит, лектин арахиса, эпителий десны, крысы.