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DYNAMICS AND MORPHOLOGY OF DENDRITIC CELLS OF THE NASAL SUBMUCOSA OF RATS' PHARYNX AFTER ANTENATAL ANTIGEN INFLUENCE

Abstract. *Researching determining mechanisms of structural and immunological homeostasis under physiological conditions, alteration of neurohumoral and metabolic control, consider to affirm that DCs are the most important modulators of skin and mucosal structure. Purpose of the study is to describe the dynamics and morphology of nasopharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus. DCs were detected on the cryostatum sections of the pharynx tissue using the Vahstein-Meisel method. DCs have high activity of ATPase. Results. It is settled that in all groups DCs number increases by 14 days of life, simultaneously with increasing of antigenic load on the body. In antigen-primed animals DCs activation take place earlier than in control, namely at the 7th day of life. In antigen-primed animals, the DCs number does not change, as compared to control, but the number of their dendrites is greater compared to control. DCs activation at 14th day of life is probably explained by an increased antigenic load on the body due to changing of the type of food. DCs high activity of ATPase is explained by activation of proteases in the endocytosis process of antigens. In conclusion, antenatal antigen influence does not cause changes in the ATP^+ DCs number in nasopharyngeal submucosa, but in antigen-primed animals, the intensity of deposition of ATP^+-material in the cytoplasm of the cells is higher and the number of dendrites in the cells is greater than that of the control and intact groups. In animals of all observed groups DCs activation passes at the first day after birth. In antigen-primed animals, the second wave of activation ATP^+ DCs takes place at the 7th day of life, namely a week earlier than in animals of the intact group.*
Keywords*: ATP, antenatal antigen action, dendritic cells, nasopharynx.*

Introduction. Dendritic cells (DCs) are the one of fundamental representations of the immune system, as they are the most efficient APCs for the activation of naive T cells. This process leads to the induction of primary immune responses. DCs reside in unflappable tissues in an immature form where they are very capable of taking up antigens but weak at stimulating T cells. After the influence of a variety of danger signals, including pathogens, proinflammatory cytokines, and dying cells, DCs undergo a process of differentiation or maturation and migrate to the T cell areas of secondary lymphoid organs [2, 5].

Researching determining mechanisms of structural and immunological homeostasis under physiological conditions, alteration of neurohumoral and metabolic control, consider to affirm that DCs are the most important modulators of skin and mucosal structure. This point is based on current data according to effects of their participation in skin and mucosal structural homeostasis maintaining. DCs participate in processes of innate and adaptive immunity, as well as in switching between immunity and tolerance depending on their activation and maturation states [1].

Purpose of the study is to describe the dynamics and morphology of nasopharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus.

Material and methods. The object of the study is pharynx of 124 white laboratory rats. Withdrawal of animals from experiment was carried out on 1, 3, 7, 14, 21, 45 days of postnatal life with decapitation. Animals are divided into 4 groups: I – intact, II – animals, which were exposed to antenatal antigen influence on the 18th day of prenatal development with the method of Voloshyn M.A. (2010) [8], III – animals, which were exposed to amniotic fluid antigen influence on the

18th day of prenatal development with the method of Voloshyn M.A. (2011) [9], IV – control animals, which were exposed to antenatal intrafetal injection of saline solution on the 18th day of prenatal development. The split virus inactivated Influenza vaccine Vaxigrip, have been used as antigen. DCs were detected on the cryostatum sections of the pharynx tissue using the Vahstein-Meisel method. DCs have high activity of ATPase, because of the activity of the ATP-dependent proton pump depends on the gradual decrease of pH in the endosomes and lysosomes, activation of proteases in the endocytosis of antigens. Other pharyngeal cells exhibit moderate to low activity of ATPase. Control of the reaction was carried out with histological preparations rich in ATPase. Preparations were contained in glycerol-gelatin. The number DCs dendrites was counted in a nasopharyngeal submucosa on a unit area of 15000 μm^2 under a microscope with oil immersion technique (ocular lens 8, objective lens 90). The variation statistics methods via program STATISTICA 6.1 was used to compare differences in number of DCs and DCs dendrites. The $p \leq 0,05$ were considered significant.

Results of study. ATP-positive DCs are found in the nasopharyngeal submucosa. At the day 1st of life in intact animals, the body of the cells has the form of an elongated triangle with spatially orientated dendrites, preferably with the button-shaped ends. DCs is present 1±0,2 at 5000 μm^2 in nasopharyngeal submucosa (Table).

The number of DCs dendrites in nasopharyngeal submucosa is 2,7±0,05 at 15000 μm^2 (Table). The absolute number of DCs ATP$^+$ in the submucosa is 1,2±0,15 at 5000 μm^2 and 1,4±0,35 at 5000 μm^2 in animals of the second and the third groups (Tabl. 1).

At day 7th of life in intact animals, the number of DCs ATP$^+$ increases in comparison with the previous observation period. The tendency of DCs number increasing in antigen-primed animals remains up to the 7th day of life. The number of DCs dendrites in the nasopharyngeal submucosa in experimental animals is statistically significantly higher compared to intact animals.

At the 14th day of life, the accumulation of ATP-positive material in the cytoplasm of DCs increases, it manifests itself by darker DCs color, also the number of dendrites increases, simultaneously their length decreases, the form changes into the fan-shaped.

In experimental animals, DCs are more vividly

Table

ATP$^+$-DCs' Dynamics in Rats' Nasopharyngeal Submucosa on the Unit Area (5000 μm^2, Vashhtein-Meisel reaction), (M ± m)

Day of Life	Group of Observation	Number of DC	Number of DC Dendrites
1	I	1±0,2	2,7±0,05
	II	1,2±0,15	4±0,13*
	III	1,4±0,35	3,9±0,08*
	IV	1±0,2	2,7±0,05
7	I	1,4±0,3	2,8±0,08
	II	1,7±0,35	3,9±0,1*
	III	1,9±0,15	4±0,05*
	IV	1,4±0,3	2,8±0,08
14	I	3±0,3#	3,4±0,08#
	II	3,2±0,5	4,7±0,23*
	III	3,4±0,35	4,6±0,08*
	IV	3±0,3#	3,4±0,08#
21	I	3,2±0,15	3±0,25
	II	3,5±0,5	4±0,05
	III	3,7±0,65	3,8±0,03
	IV	3,2±0,15	3±0,25
45	I	3±0,3	2,6±0,18
	II	3,4±0,35	3,5±0,1
	III	3,5±0,5	3,5±0,05
	IV	3±0,3	2,6±0,18

*Notes: I – intact, II – animals, which were exposed to antenatal antigen influence, III – animals, which were exposed to amniotic fluid antigen influence, IV – control animals; the symbol * means that the result is statistically probable with respect to the intact group, the symbol # means that the result is statistically probable in relation to the previous observation period.*

colored throughout all observation periods than in control. Most often there are cells with enormous number of dendrites than in common DCs, having a predominantly fan-shaped form. In experiment the DCs dendrites are visually thicker and more intense coloring than in control. The content of DCs in the submucosa throat in experimental rats tends to increase compared to the intact group.

During the period from the 21 to the 45 day of life in intact animals, intense deposition of ATP-positive material in the DCs cytoplasm doesn't change, the shape of the dendrites is predominantly fan-like. DCs number is at the level of the previous observation period. In antigen-primed animals at the 21st day of life, there the DCs number is higher compared to control, there are cells with a greater number of short dendrites (Tabl. 1). The shape of dendrites is different: fan-shaped endings prevail, except them button-

shaped dendrites are also present.

Discussion. DCs number increases throughout first two weeks, simultaneously with increasing of antigenic load on the body. In antigen-primed animals, regardless of the input method of antigen, DCs activation take place earlier than in control, namely at the 7th day of life. In antigen-primed animals, the DCs number does not change, as compared to control, but the number of their dendrites is greater compared to control. DCs activation at 14th day of life is probably explained by an increased antigenic load on the body due to changing the type of food.

DCs high activity of ATPase is explained by activation of proteases in the endocytosis process of antigens. Adenosine is a well-studied neurotransmitter, but as a part of ATP it also exerts profound immune regulatory functions. Extracellular ATP acts as a “danger” signal and stimulates immune responses, i.e. by inflammasome activation [6]. Its degradation product adenosine also acts rather anti-inflammatory, as it down regulates functions of DCs and dampens T cell activation and cytokine secretion [3]. DC derived adenosine can also act back onto the DCs in an autocrine manner. This leads to suppression of DCs functions that are normally involved in stimulating immune responses. Moreover, ATP and adenosine production thereof acts as “find me” signal that guides cellular interactions of leukocytes during immune responses [4, 7].

The more ATP is accrued in DCs, the more credibly DCs will derivate adenosine. It is consequents to suppresses activation of T cells. Also derivation of adenosine can preventing DCs maturation and development of effectors’ functions. This probably leads to decrease in the reactions of local immunity.

Conclusions. Antenatal antigen influence does not cause changes in the ATP^+ DCs number in nasopharyngeal submucosa, but in antigen-primed animals, the intensity of deposition of ATP^+-material in the cytoplasm of the cells is higher and the number of dendrites in the cells is greater in control. In animals of all observed groups DCs activation passes at the first day after birth. In antigen-primed animals, the second wave of activation of ATP^+ DCs takes place at the 7th day of life, namely a week earlier than in animals of the control and intact groups.

Prospects for further research. It is planned to describe the dynamics and morphology of oropharyngeal submucosa DCs in the postnatal period after the antenatal antigen action on fetus.

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