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MICROELEMENT PROFILE AND STRUCTURE OF REGIONAL LYMPH NODES DURING SENILE INVOLUTION OF LYMPHOID TISSUE

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ABSTRACT — The microelement profile and structure of lymph nodes were studied in experiment using old Wistar rats by means of a morphological method and the X-ray fluorescent analysis with synchrotron radiation. Their special qualitative and quantitative status is formed in regional lymph nodes that reflect age-related dynamics of lymphoid tissue. The content of trace elements and the size of compartments differ in lymph nodes of different localization. Age-related modifications in a microelement profile are characterized by proliferation of lymphoid cells, development of compartments and initiation of a corresponding immune response at the level of regional lymph nodes with different contact to external environment. Localization of lymph nodes is one of conditions for realization of the principle of a regional determinant (regional specifics).

KEYWORDS — morphology, lymph nodes, trace elements, gerontology.

INTRODUCTION

Expansion of knowledge of a role of chemical elements (bioelements) and lymphatic system became a basis for integration of a medical bioelementology [1] and a lymphology [2, 3]. Changes in homeostasis of trace elements and the lymphatic system accompany various physiological and pathologic conditions including aging of an organism [4, 5, 6]. Lymph nodes continue to be in the center of attention of lymphologists because of their protective function during the different periods of life. Studying the microelement profile of peripheral lymphoid organs as components of lymphatic regions is relevant. Assessment of participation of essential trace elements in forming of the immune status of lymph nodes belongs to unresolved questions though microelements have immunotropic properties [4, 7]. It is necessary to reveal relationships of cause and effect of trace elements content and structure of regional lymph nodes.

The purpose is to characterize modifications in content of trace elements and the structural organization of regional lymph nodes in aging.

MATERIALS AND METHODS

The experiment was carried out on white Wistar rats aged 1.5–2 years (old animals). Mesenteric, inguinal and tracheobronchial lymph nodes were investigated by a morphological method. Lymph nodes were fixed in 10% neutral formalin. After fixing we used the standard scheme of washing, dehydrating, embedding in xylene and paraffin. After that the microtome was operated to prepare histologic sections. Finally, the histologic sections of lymph nodes were stained with hematoxylin and eosine, azure-II-eosine and Masson's trichrome staining.

The choice of Se, Mn, Fe, Cu, Zn trace elements can be explained by the fact that they are essential bioelements which support the immune system in accordance with the modern classification [1]. The content of trace elements in lymph nodes was defined by the X-ray fluorescent method with the use of synchrotron radiation. The work was done on the equipment of Budker Institute of Nuclear Physics (Novosibirsk), supported by project RFMEFI62119X0022 [8].

The morphometric analysis of lymph node structures was carried out by means of a morphometric grid and the Image-Pro Plus 4.1 program. Statistical data processing was performed with licensed statistical software package StatPlus Pro 2009, AnalystSoft Inc. The semiquantitative method was used for assessment of data. A P-value < 0.05 was considered statistically significant.

RESULTS

It is established that there are age-dependent fluctuations of exchange of trace elements [4, 5, 9]. The concentration of bioelements varies not only in organs, but also in regional lymph nodes. In majority of trace elements concentration reduces by 1.2–1.5 times ($p < 0.05$–0.01) compared to the maximum possible

value at young age [6]. We noted that the differences in bioelements content of lymphoid tissue depend on the extent of their accumulation of different localization (regional specifics) in lymph nodes. There are higher rates of content of Mn, Fe, Zn and minimum for Se in an inguinal lymph node. There is a high value concentration of Se (1.14±0.06 mkg/g) in the mesenteric lymph node. The content of selenium exceeds by 1.4–1.5 times concentration in tracheobronchial ($p < 0.001$) and inguinal ($p < 0.05$) lymph nodes. There are low values of content of Mn, Fe, Zn and Se in the tracheobronchial lymph node.

Level the content of Cu lies in the interval 4.68±0.27–5.37±0.14 mkg/g in lymph nodes of different topographical groups. Within this interval the smallest content of Cu is observed an inguinal lymph node, the greatest one is noted in the tracheobronchial lymph node (Table 1). Comparison of microelements content revealed reliable differences between lymph nodes, belonging to lymphatic regions that are located in different contact with the external environment. Accumulation and removal of microelements happens unequally in lymph nodes that leads to forming individual qualitative and quantitative microelement profile in aging.

Table 1. *The Microelement Status of Lymph Nodes in old age period*

Trace elements	Inguinal lymph node	Mesenteric lymph node	Tracheobronchial lymph node	P
	1	2	3	
Mn	+++	+	++	$P_{1-2} < 0{,}001$ $P_{1-3,\,2-3} < 0{,}05$
Fe	+++	+	++	$P_{1-2,\,2-3} < 0{,}05$ $P_{1-3} > 0{,}05$
Zn	+++	++	+	$P_{1-3} < 0{,}001$ $P_{1-2,\,2-3} < 0{,}01$
Cu	+	++	+++	$P_{1-3} < 0{,}05$ $P_{1-2,\,2-3} > 0{,}05$
Se	+	+++	++	$P_{1-2} < 0{,}001$ $P_{1-3,\,2-3} < 0{,}05$ $P_{2-3} > 0{,}05$

The bioelemental composition reflects specific features of the structural and cellular organization of lymph nodes in aging. There is a prevalence of medullary substance mainly at the expense of a B-dependent zone (medullary cords) and evenly expanded lymphatic sines at minimization of lymphoid compartments in the mesenteric lymph node (Table 2). Such structure of a lymph node defines the immune response of humoral type. Trace elements together with enzymes make a certain contribution to proliferation of lymphoid (immune) cells and to antioxidant protection [1, 4, 7]. It is provided with the different level of accumulation of trace elements. We noted the maximum contents Se, an average — Cu, Zn and minimum — Fe, Mn (Table 1). The distinction of concentration of trace elements defines extent of proliferative processes, development of compartments and leads to formation of the immune response of humoral type in the mesenteric lymph node.

Table 2. *The morphological status of lymph nodes in old age period*

Lymph nodes sructures	Inguinal lymph node	Mesenteric lymph node	Tracheobronchial lymph node	P
	1	2	3	
Lymphoid follicles	+	+	+	$P_{1-2,\,1-3,\,2-3} > 0{,}05$
Cortex plateau	++	+	+++	$P_{1-2,\,1-3} < 0{,}05$ $P_{2-3} < 0{,}01$
Paracortex	+++	+(+)	++	$P_{1-2,\,1-3} < 0{,}01$ $P_{2-3} > 0{,}05$
Medullary cords	++	+++	++	$P_{1-2,\,2-3} < 0{,}01$ $P_{1-3} > 0{,}05$
Lymphatic sinus	+++	++	+	$P_{1-2,\,2-3} < 0{,}05$ $P_{1-3} < 0{,}001$

There is an expansion of the area of an interfollicular part of cortex and medullary cords (B-zone), a paracortex (T-zone) against the background of narrow lymphatic sine in the tracheobronchial lymph node (Table 2). The immune response of the mixed type is formed at simultaneous representation of cortical and medullary substances at a limited drainage function. The morphoimmune status of a tracheobronchial lymph node is combined with the maximum contents of Cu, an average — Se, Mn, Fe, minimum — Zn (Table 1).

There is a rather wide T-dependent zone at the expense of a paracortex and an interfollicular part of cortex at wide lymphatic sines in an inguinal lymph node (Table 2). The immune response of cellular type is formed in an inguinal lymph node. The inguinal lymph node distinguishes the maximum content of Mn, Fe, Zn and minimum — Se, Cu (Table 1). The

content of Zn Fe is a priority. These trace elements are essential for normal division, proliferation and a differentiation of T-cells [10, 11] and for further development of a paracortex with the immune response of cellular type.

The discretization of structural units of lymph nodes defines vulnerability of elements of a system at age-dependent changes. In old animals there is a reduced number of lymphocytes, lymphoblasts in compartments of lymph nodes including in lymphoid follicles. It is necessary to assume that the micronutrient deficiency, in aging, affects the activity of enzymes that results in decrease of cellular proliferation and a reduction of lymphoid follicles [4, 5, 7].

Lymphoid follicles reach the smallest sizes within 4.79±0.27%–5.84±0.36% without demonstration of statistically reliable difference between lymph nodes ($p > 0.05$). There is a reduction of a lymphocytic pool due to decrease of immunogenesis. It is obvious that forming of a certain microelement profile and morphotype of lymph nodes depend on localization of the lymphatic region and features of contact with external environment. Lymphoid follicles are a systemically important element and as reactive structures are responsible for a lymphopoiesis and forming of compartments. Trace elements enter cofactors of many enzymes and they are necessary for proliferation and functioning of lymphoid (immune) cells. Age-dependent changes are characterized by correlation ratios between the content of microelements and the area of lymphoid follicles.

Thus, lymphoid follicles without the germinative center show positive correlation concerning Mn ($r=0.34$, $p < 0.05$) and Se ($r=0.52$, $p < 0.01$) in the tracheobronchial lymph node. There is negative correlation concerning Fe ($r=-0.30$, $p < 0.05$) and Zn ($r=-0.45$, $p < 0.01$) in an inguinal lymph node. There is a positive correlation with Mn ($r=0.65$, $p < 0.001$) and Fe ($r=0.48$, $p < 0.01$) in the mesenteric lymph node. Lymphoid follicles with the germinative center are most indicative. There is a positive correlation of lymphoid follicles with the germinative center concerning Cu ($r=0.33$, $p <0.05$) in the tracheobronchial lymph node. We noted weak degree of correlation ($r <0.30$, $p> 0.05$) for all trace elements in inguinal and mesenteric lymph nodes. The correlation between trace elements and lymphoid follicles does not allow to speak about existence of steady lymphoid-microelement association.

We, for the first time, described the formation of lymphoid-microelement association during the maximum development of lymphoid tissue [6, 9]. Reduction of activity of lymphoid follicles and decrease of correlation with microelements can disrupt lymphoid-microelement association during age-related changes of lymphoid tissue. Possible deviations are the result of temporary disadaptation or a readaptation of a structurally functional condition of lymph nodes connected with their localization and age.

DISCUSSION

Aging of an organism affects all organs and systems including lymphoid tissue. Pathognomonic sign of aging is involution of lymphoid tissue with its replacement by fatty or connective tissue [2]. Nevertheless many peripheral lymphoid organs (lymph nodes) keep the structure, but it has features of transformation in aging [6, 9]. We noted interrelation of microelements with the morphoimmune status of lymph nodes of different localization. Immune function of lymph nodes is reflection of development of compartments in the course of proliferation and a differentiation of immunocompetent cells. Trace elements influence cells of the lymphocytopoietic system of lymph nodes and as a response modify the area of compartments of lymph nodes.

The immunogenesis is reduced and there is a reduction of a lymphocytic pool in combination with the low content of trace elements in old animals [7, 9]. Concentration of separate trace elements differs in regional lymph nodes. A certain microelement profile and the corresponding morphotype in each of the studied lymph nodes provide the immune response of humoral type in the mesenteric lymph node, the mixed type in the tracheobronchial lymph node and cellular type in an inguinal lymph node (regional specifics). The principle of a regional determinant is implemented according to the concept of the lymphatic region by Yu.I. Borodin [3], there are reliable differences between lymph nodes, belonging to various lymphatic regions. Each of lymphatic regions differently contacts to external environment, as causes age-dependent originality of lymph nodes.

CONCLUSION

The obtained results demonstrate specifics of trace element exchange and the structural organization of the regional lymph nodes natural aged. The principle of a regional determinant is the basic in formations a microelement profile and a morphoimmune status of lymph nodes of different localization according to the concept of the lymphatic region. The indispensable condition of functioning of lymph nodes is existence of trace elements in a certain concentration. Trace elements influence process of proliferation and a differentiation of immunocompetent cells, development of compartments and variant of the immune response in lymph nodes of different lymphatic regions. Structural features and a microelement profile of lymph nodes

serve as a prognostic tool for aging of peripheral lymphoid organs. The results can be employed to correct balance of trace elements and to increase the protective function of lymph nodes at the stage of senile involution in lymphoid tissue.

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