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СТАН ІМУННОЇ СИСТЕМИ ШЛУНКОВО-КИШКОВОГО ТРАКТУ ЩУРІВ У ЗМІНЕНИХ АНАТОМІЧНИХ УМОВАХ

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Реферат

Червоподібний паросток, лімфатичні вузли кишківника, лімфоцити слизової оболонки утворюють лімфоїдну тканину шлунково-кишкового тракту (GALT - англ. Gut-Associated lymphoid tissue). Лімфоцити шлунково-кишкового тракту мають дві основні функції: продукція IgA, який є першою лінією імунного захисту і регулюють процеси імунологічної відповіді на антигени, які потрапляють у шлунково-кишковий тракт. З метою кращого вивчення будови та функціонування системи GALT проведено дослідження на 20 статевозрілих самцях білих щурів лінії Вістар. Тварин розділили на дві групи А і В, кожна з яких налічувала по 7 щурів, і одну контрольну групу К, яка складалася з 6 тварин. Щурам однієї групи видалили червоподібний паросток, а іншої групи - усі лімфатичні вузли ілео-цекального кута. Щурам контрольної групи робили лише лапаротомію. Після 63 днів прижиттєво у щурів видалили відповідні органи та вивели тварин з експерименту шляхом декапітації. Червоподібні паростки та лімфатичні вузли ілео-цекальної ділянки були досліджені макро- та мікроскопічно та порівняні із контрольною групою. Не виявлено жодних морфологічних відмінностей. Проведено також імуногістохімічне дослідження із застосуванням антитіл Mous Anti Rat CD 45 RA до всіх лімфоцитів В, а також Mous Anti Rat CD 43 до всіх лімфоцитів Т та оцінено інтенсивність імуногістохімічної реакції із застосуванням комп'ютерного аналізатора. Встановлено, що у паренхімі лімфатичних вузлів ілео-цекальної ділянки тварин дослідної групи зменшилася середня кількість лімфоцитів В, середня кількість лімфоцитів Т не змінилася. Внаслідок цього змінилося співвідношення лімфоцитів В до лімфоцитів Т. Подібні імунологічні зміни можуть спостерігатися у інших органах імунної системи, тому необхідно продовжувати дослідження та вивчати зміни.

Ключові слова: *GALT, імунна система, апендектомія, морфологія, лімфатичні вузли*

Abstract

A STATE OF ALIMENTARY TRACT IMMUNE SYSTEM IN RATS UNDER ANATOMICALLY CHANGED CONDITIONS

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Appendix, solitary and intestinal lymph follicles, mucous lymphocytes make a cluster of gut- associated lymph tissue called GALT. Alimentary tract lymphocytes provide two basic tasks; they produce Ig A being the first defence line against pathogenic micro-organisms and regulate processes of immunologic response to antigens reaching its lumen. To recognize GALT structure and its function better, the experimental study on 21 sexually mature male rats of Wistar strain was carried out. The animals were divided into 2 study groups A and B each consisting of 7 rats and 1 control K consisting of 7 rats. The rats from one study group A had an appendectomy and the rats from the other group B had ileocolic lymph nodes removed. The rats from the control group K underwent only laparotomy. After 63 days, the respective organs were taken and the animals were sacrificed. The appendices and ileocolic lymph nodes functioning for 63 days in the anatomically changed conditions underwent macro- and microscopic examination and were compared wih the image of the control group organs. No significant morphologic differences were found. Additionally, immunohistochemical examination was carried out with specific antibodies Mous Anti Rat CD 45 RA aimed against all B lymphocytes and Mous Anti Rat CD 43 aimed against all T lymphocytes and the evaluation of the immunohistochemical reaction intensity was done with the use of a computer image analyzer. A decreased average number of B lymphocytes was found in the ileocolic lymph node parenchyma of the experiment animals; mean number of T lymphocytes was on the same level. The quantitative ration of B lymphocytes to T lymphocytes changed. Similar immunologic changes can be present in other immune system organs; therefore, it seems necessary to continue the experiments and study.

Key words: *GALT (gut associated lymphoid tissue), immune system, appendectomy, lymph nodes, lymphology*

Introduction

More common use of imaging examinations in diagnosing acute disorders of abdominal cavity makes the number of unnecessary appendectomies smaller. Still, according to contemporary studies, as much as 15-30% of the removed appendices do not show any pathological changes. Removing an unchanged appendix

is always connected with the risk of complications and generates considerable costs [1]. So far, little is known about the influence of appendectomy on the mucosa immune efficiency of MALT (mucosa-associated lymphoid tissue). Although it has been known for quite a long time that the appendix wall contains a cluster of lymphatic tissue, it is often defined as a vestigial organ. The appendix is a part of GALT (gut-associated lymphoid tissue) which provide the immune protection against micro-organisms penetrating with food into alimentary tract. The huge surface (c. 100 m^2) of the inner organism environment, which is the alimentary tract wall, getting into contact with the outside world, requires great efficiency of immune system. Additionally, this is the surface of absorption process. Therefore, the population of gut lymphocytes is so numerous that it can be compared to the spleen's lymphocyte number, the biggest in human organism [4].

GALT system, as well as the whole lymphatic system fulfils its role by creating immunity or immune tolerance. As a result, pathogenic germs and mild antigens get into the alimentary tract with food; immune mechanisms are usually activated against the germs, while the antigens trigger off the tolerance phenomenon [5]. Both the insufficiency or lack of immunity against dangerous invaders and weakening or loss of tolerance against mild or own antigens are dangerous for the organism [6].

The appendix wall has 4 layers like an intestine. Lymph follicles are located mainly in the lamina propria of the musoca and also in the submucosal membrane creating its primary mass. Appendix epithelium has a character of lymphoidal intestinal epithelium [7]. Likewise, in lymph nodes and intestinal lymph follicles, high endothelial venules (HEV) are located, through which lymphocytes flowing with blood reach their destination [8].

All the appendix lymph follicles are surrounded by wide lymphatic sinuses. These sinuses connect between themselves and with the net of capillary lymphatic vessels. Lymph flows away through lymphatic vessels of mesoappendix directly to ilecolic lymph nodes. Appendix in humans is a narrow, blindly ended, digitate bulge of caecum wall. In rats, caecum is relatively longer than in people and much wider than the colon; appendix is connected to it without any clear border and is relatively larger than in humans [9]. Ileocolic lymph nodes make a clustered group below ileum terminale and correspond topographically and functionally to human ileocolic lymph nodes. Usually, up to three individual nodes are observed. Sometimes, there is only one large node. The parenchyma of those nodes has three clearly outlined topographic areas and a structure characteristic for mesenteric nodes. Numerous and big lymph follicles containing proliferation centres can be seen in superficial cortex in a microscopic image. Several big entities can be seen in the deep cortex [10]. The lymph flowing from the appendix area into the ileocolic lymph nodes flows directly from the alimentary tract wall, while in people, an indirect route through tiny nodes of mesoappendix is possible [11]. A node inner structure is conditioned by several factors. They are congenital or acquired factors depending on its functions. Size and shape depend on its inner structure. Morphologic differences of the structures being a part of node individual physiologic compartments can be explained by the fact that every vessel can carry lymph containing various immune factors. Several afferent vessels from the ileum terminale, caecum and appendix lead into the ileocolic lymph node. These organs make the subsequent, adjacent parts of alimentary tract. Therefore, this lymph node can be defined as a relatively mono-organ node. In normal conditions, lymph flows from GALT organs to mesenteric lymph nodes. The one-way lymph flow indicates that mesenteric nodes are a place where the cooperation between GALT and systemic immune system occurs [12].

As practice shows, one can live without an appendix. However, it is not known exactly how its removal influences other GALT structures and systemic immune system, especially in biologically immature individuals. Some authors call the appendix an intestinal tonsil; others compare its role with the role of lymph follicles concentrated around the intestine. There is also an opinion that the appendix is an equivalent of Fabricius bursa in rodents [13]. The purpose of the studies was to clear up whether appendectomy in rats brings any morphological changes in ileocolic lymph nodes and disturbs their structure and function in the period of 63 days.

Material and Methods

Experimental study was performed on 21 sexually mature male rats of Wistar strain according to the permission granted from the ethical commission. The average rat's age was 3 months, weight from 200 to 250 g. The rats came form the Central Experimental

Animal Farm of the Silesian Medical University in Katowice. Animals were kept in the same standard conditions during the entire experiment. All the operations were performed in the operation theatre of the Animal Farm in the standard conditions, assisted by the veterinary doctor who anaesthetized the animals. All the animals were operated by opening the peritoneal cavity with the median incision. The animals from the control group K had a sham operation, i.e. only laparotomy was done.

The animals from group A had an appendectomy and the animals from group B had all the ileocolic nodes removed. All the animals were sacrificed after 63 days, after taking samples of appendices and lymph nodes in the control group and lymph nodes in the group A and appendices in B. The samples were examined macroscopically. Next, the appendix and lymph nodes of an individual rat were placed separately in a marked containers and were fixed in 10% buffered formalin and inserted in paraffin. Paraffin blocks were cut into sections of 5 micrometers. The sections were dyed with hematoxylin and eosin. Lymph structures of the organs were analyzed and the received results were compared with the organs from the control group K.

Microscopic evaluation was done according to the concept of Sainte-Marie, Belisle and Penga [13,15]. The evaluation mainly included the number and size of superficial cortex lymph follicles, number and shape of the items of deep cortex and width of sinuses and spinal cord column. The observation in the images of the appendix focused mainly on the number and size of lymph follicles and their distribution in the lamina propria of the mucosa and in the submucous membrane. The thickness and continuity of this layer was analysed. The immunohistochemical test was performed with the use of specific antibodies of Serotec company: MCA 340R Mouse Anti Rat CD 45 RA (IgG) concentration 1 mg/1ml (working concentration 1:100) and MCA 54G Mouse Anti Rat CD43 (IgG) concentration 1mg/1ml (working concentration 1:10). Antigen-antibody reaction was shown using the LSAB2/HRP method, DAB set of DAKO company, catalogue no KO 675 was used. DAB set of DAKO company, catalogue № K3468 was used as chromogen for peroxidase, which as a result of oxidation gives a brown product of the reaction. Cell nuclei were stained with Mayer's hematoxylin. Immunohistochemical dyeing was done in 10 stages. The evaluation of the immunohistochemical reaction intensity was done in pixel units with the use of the computer image analyzer KS-400, Kontron company. The system was equipped with a research microscope Axioplan2 with a motorized table and coloured camcorder 3CCD, by Sony. Picture acquisition was done by the 5X lens enlargement. The automatic single image of the whole cross-section of the investigated nodes was generated automatically from the adjacent 25 fields. Such an image was archived and analysed. At the first stage, a median filter was used, and then region mask was generated by the threshold function. Region selection was done manually. The total area of the selected regions was calculated for each field. The area occupied by the tissue of strong antigen expression in relation to the total cross-section of the examined node was calculated as a final result. The results are presented in tables. The values are given in pixels.

Results and Discussion

A macro and microscopic picture of appendices and lymph nodes in the animals from group A and B did not show any significant differences as compared to the organs of the animals from the group K. Two ileocolic lymph nodes of similar size were found in each individual. In some animals, the nodes were more spherical than oval of 3 mm diameter. They were of white colour, and the surface was smooth and slightly crenated. Similarly, no differences in quality were found in immunohistochemical examinations. In lymph nodes of animals from all the groups, CD43 positive lymphocytes (pan-T) were found mainly within the units of deep cortex, where they formed dense system. In the intrafollicular sphere of superficial cortex, they were similarly numerous and densely distributed. The intrafollicular sphere smoothly connected with the units of deep cortex. T lymphocytes in the lymph follicles were distributed peripherally in a quite dense structure, while in the central part as individual cells. Similarly, dense structure of T cells in the peripheral part of lymph follicles was observed in spinal cord columns. T lymphocytes filled the subcapsular and trabecular sinuses in large numbers; a smaller number was observed in medullar sinuses. CD 45RA positive lymphocytes, i.e. B lymphocytes, were present in great numbers in the superficial cortex of the lymph nodes. They made an especially dense structure around the periphery of lymph follicles, while they were more scarce in the middle part and in the intrafollicular sphere which connected smoothly with a unit of deep cortex.

B lymphocytes were as densely located in the peripheral part of the deep cortex unit as in the intrafollicular sphere of superficial cortex. On the other hand, their less denser distribution was observed in the middle unit of deep cortex. Spinal cord columns did not separate clearly from deep cortex units and the intrafollicular sphere of superficial cortex. Big cluster of B lymphocytes was seen in the subcapsular and trabecular sinuses . Similarly, the abundant number of B cells was observed in medullar sinuses. The abundance of B and T lymphocytes in subcapsular and trabelcular sinuses was similar; however, a clearly larger number of B lymphocytes compared toT lymphocytes was seen in medullar sinuses.

The length, lumen width, and wall thickness of the appendices were estimated. All the appendices, which is characteristic for rats, were relatively big. Their length was from 45 to 60mm, lumen width ranged from 3 to 5 mm, and their wall was about 2 mm thick. CD 43 lymphocytes, i.e. pan-T lymphocytes, were observed in dispersion in the parenchyma of lymph tissue cluster in the appendices of animals from all the groups. A small number of cells was distributed irregularly within each lymph follicle. There were more of them and they made much denser structure in the vicinity of sinuses, in the intrafollicular sphere. Individual T cells were found among the intestinal epithelium cells. CD 45RA positive lymphocytes, i.e. B lymphocytes, observed in dispersion in the parenchyma of appendix lymph tissue cluster made much denser and more abundant structure than T lymphocytes. They were distributed evenly within the lymph follicles and among them. They were quite numerous in the intestinal epithelium.

However, some differences were found in immunohistochemical quantitative evaluation. PD/PS ratio is a ratio of the area taken by the tissue of strong CD43 antigen expression to the total cross section of the studied lymph node measured in pixels, which reflects the number of T lymphocytes in the investigated cross sections and a ratio of PD/PS for CD45RA antigen, which reflects the number of B lymphocytes in the investigated lymph nodes cross sections of the animals from group A and control group were taken into consideration. To compare the number of T and B lymphocytes in the nodes of both groups, the ratio of PD/PS CD43 to PD/PS CD 45RA was calculated. The average in the control group nodes was 0.8214, and in the study group A - 1.0760, which is slightly higher.

Investigating the macroscopic and microscopic structure of the selected rat's lymph organs with both classical methods and with monoclonal antibodies, no significant morphological differences between the organs of control and study group animals were found. Morphology of ileocolic lymph nodes, 63 days after appendectomy, did not show any qualitative changes. It can be noticed that no visible morphological changes appeared within the lymphatic organs functioning for 63 days in the anatomically changed conditions.

No signs of proliferation in their parenchyma, nor excitation or atrophy were observed. However, using computer analyzer allowed to discover certain differences in quantitative character , which can indicate a functional change within the investigated ileocolic lymph nodes of rats whose appendices were removed 63 days before. A mean number of B lymphocytes clearly decreased as compared to the control group nodes. At the same time, the ratio of the number of B lymphocytes to T lymphocytes changed in those animals nodes, which seems to be of some importance for the node function. This can be an evidence of a disturbed balance and cooperation between B and T lymphocytes in the process of immunological response. However, in the light of the available knowledge the implications and meaning of this fact remain undetermined.

It corresponds in an interesting manner with the results of the experimental study made by Watson et al. [16], where the authors decisively underline the role of the appendix in creating mucosal immunity. According to them, removing an unchanged appendix, especially in young individuals in states of abdominal infections, such as mesentric lymphadenitis , can protect against future development of ulcerative enteritis or Crohn's disease. The authors concentrated more on quantitative changes. They did not investigate ileocolic lymph nodes of the operated animal but they showed the changes in T lymphocytes subpopulation in the walls of the removed appendices. In their study, T cytotoxic lymphocytes were present in all removed appendices, while T auxiliary lymphocytes were present in the most perforated inflammatory appendices, but only in half of the inflammatory but unperforated appendices.

Many researchers pay attention to special importance of micro-organisms living in the colon and the role of secreted exogenous ligands in creating proper environment for lymphocyte maturation within GALT. Appendix, because of its location and structure, is a very specific organ. Among all the GALT organs, it is

the only organ located on the border of the small and large intestine, which causes it, not the Peyer's patches, to have better contact with microorganisms of the colon. Some authors performing experiments on rabbits think that growth, differentiation and selection processes of newly-grown B lymphocytes in young rabbits take place in appendix; therefore , they claim that the appendix in mammals is an equivalent of bird's Fabricius bursa [13]. In rats at large and in humans, the appendix contains large cluster of lymph tissue which creates continuous and thick layer in submucosal membrane. In our study group, the attention was drawn to an agglomeration of lymph follicles in the appendix wall both in control and experimental group. It could be a result of immunomoduling reaction of steroid hormones, the concentration of which in blood increases considerably in the post-puberty period, since the rats in the experiment were sexually mature [17]. The small concentration of lymph tissue in laboratory rats' appendices might be connected with their diet of standard rat's feed which is clean bacteriologically and, as a foodstuff of mainly vegetarian origin, has less stimulating effect on GALT structures. Bigger amounts of protein and fat as well as micro-organisms present in natural feed provide an antigen burden to the immune system. It is confirmed by the experiments on germfree animals. Not only GALT of those animals but also lymph nodes and thymus have a different structure [18].

The basic role of each lymph node is to purify the lymph from unnecessary ingredients regardless of their immunogenic character . Immunogenic particles present in the lymph stimulate various structures constituting the lymph node. Experiments on rats carried by Sainte-Marie et al. [19] allowed to determine that the route of lymph flow through a node has several variants and depends on inner architecture of each node, primarily on whether superficial cortex in a node and subcapsular sinus located above make a continuous layer or are segmented. The nodes with continuous surface and sinus are called nonsegmental, and those in which superficial cortex and subcapsular sinus have characteristic breaks are called segmental. All the investigated ileocolic lymph nodes were segmental; therefore, the route of lymph flowing through them did not differ, which could have some influence on the condition of the lymph and parenchyma.

The lymph flowing through the node has a transport and mechanical function. The mechanical function lies in rinsing away the lymphocytes gathering in the node sinuses. After binding up some afferent node vessels, the overfilling of medullar sinuses of those nodes with a great number of lymphocytes can be observed. This phenomenon occurs on the first day after the operation and remains until a collateral circulation is created [20]. During the experiment, it was important to remove the appendix together with the whole mesentery. The fact is that ileocolic nodes are not mono-organ nodes since they receive lymph not only from the appendix but also from the ileum terminale and the colon. Therefore, general atrophy of cortex medullar structures of those nodes could not have been expected after appendectomy.

Interestingly, not even the smallest signs of atrophy were observed in the parenchyma of the investigated nodes. However, one can suspect that the amount of the flowing lymph in the anatomically changed conditions had to decrease. As the studies of Olszewski et al. [21] show, lymphatic collateral circulation is created already 7 days after cutting or removing the afferent lymph vessels. In case of appendectomy in this experiment, it could not be created because the whole organ was removed with lymph vessels. Or perhaps, a uniform organ character of the ileocolic node drainage area, which allows to qualify this node in the group of relatively mono-organ nodes, provides the conditions for creating connection between the cut out afferent node vessels and peripheral vessels of the remaining organs of the drainage area.

Nevertheless, the fact that no sinus overfilling in the parenchyma of all active lymph nodes in the anatomically changed conditions was observed is an evidence that the amount of the lymph flowing into nodes was sufficient. It is also possible that the antigen stimulation of ileocolic node after appendectomy remains on the same level as a result of compensation and limiting the drainage area, if the appendix can be considered equal in its function to Peyer's patches.

Ileum terminale is still a place of a specific cluster of lymph follices similar in their structure to those observed in the appendix. No visible symptoms of cessation of lymphocytes circulation, no signs of proliferation or atrophy were observed in the parenchyma of the investigated animals' lymph nodes which had appendectomies 63 days before. No distinct morphological changes within the investigated lymph nodes which are in the closest vicinity makes us wonder if possible changes should be searched for in other lymphatic organs, e.g. spleen? On the other hand, a

presence of certain quantitative changes in node parenchyma brings a question whether changes after appendectomy within GALT or systemic immune system are not only of functional character.

Conclusions

Appendectomy in rats did not result in visible morphological changes of ileocolic lymph nodes. The only changes observed were immunological changes in the nodes parenchyma. The mean number of B lymphocytes decreased as compared to the ileocolic lymph nodes of control group animals, which could be a symptom of functional changes occurring in those organs. No morphological changes in the investigated organs do not exclude a presence of changes in other organs belonging to GALT or to systemic immune system. The immunological changes observed in ileocolic lymph nodes can also be present in other lymphatic organs. To recognize the biologic role of appendix, further studies should be performed.

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