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Functional zones of Peyer's patch (PPs) in rabbits' caecum

M. O. Nikitina, M. V. Kravtsova, A. A. Bohomaz
Dnipro State Agrarian and Economic University, Dnipro, Ukraine

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Dnipro State Agrarian and Economic University, Sergii Efremov Str., 25, Dnipro, 49600, Ukraine

Tel.: +38-067-107-77-78
E-mail: nikitina.m.o@dsau.dp.ua

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Abstract. A feature of rabbit gut-associated lymphoid tissue is that its structure is more developed than in other animal species. In rabbits it is composed of sacculus rotundus, vermiform appendix and Peyer's patches. These immune formations contain an organized component of lymphoid tissue – lymphoid nodules (B-cell zone) and interfollicular region (T-cell). Secondary lymphoid nodules with germinal centers presented in them are formed due to antigen stimulation. The caecum of Hyplus rabbits at the age of 30 -, 60 - and 90-days was investigated. Each age group consisted of 5 rabbits. Experimental rabbits are clinically healthy, unvaccinated and untreated against ecto- and endoparasites. Peyer's patches of the caecum were selected for the study and fixed in 10% of formalin. Subsequently, the specimens stained with hematoxylin-eosin were prepared from the obtained samples. On the 30th day of life, Peyer's patches in the cecum were detected by gross examination. On the histological level, they had formed interfollicular region and lymphoid nodules. In turn, lymphoid nodules were divided into primary and secondary ones. A well-defined mantle zone and germinal centers were observed in the secondary lymphoid nodules. The regularities of their area indicators increase (mean value, median and interquartile range (IQR)) and their correlation were studied. The most intensive growth of the mantle area and the germinal center was observed from the 30th to the 60th day. The relative area of the mantle zone and the germinal center as part of the secondary lymphoid nodule was determined. Its value did not change during the experimental period.

Keywords: lymphoid tissue; lymphoid nodules; germinal centers; mantle zone; interquartile range

Функціональні зони плямки Пейєра сліпої кишки кролів

М. О. Нікітіна, М. В. Кравцова, А. А. Богомаз
Дніпровський державний аграрно-економічний університет, Дніпро, Україна

Анотація. Особливість лімфоїдної тканини, асоційованої з кишками у кролів полягає в тому, що вона більш розвинута, ніж у інших видів тварин. У кролів до її складу входять лімфоїдний дивертикул, червоподібний відросток та плямки Пейєра. Ці імунні утворення містять організовану складову лімфоїдної тканини – лімфоїдні вузлики (В-клітинна зона) та міжвузликову зону (Т-клітинна). Внаслідок антигенної стимуляції формуються вторинні лімфоїдні вузлики з гермінативними центрами. Досліджено сліпу кишку кролів породи Хіплус 30-, 60- та 90-добового віку по п'ять особин у кожній віковій групі. Дослідні кролі клінічно здорові, не підлягали вакцинації, обробці від екто- та ендопаразитів. Для дослідження відібрані плямки Пейєра сліпої кишки, зафіксовані у 10% формаліні з подальшим виготовленням гістопрепаратів із забарвленням гематоксилін-еозином. Встановлено, що на 30-ту добу життя макроскопічно виявляли плямки Пейєра у сліпій кишці. На гістологічному рівні вони мали сформовані міжвузликовий простір та лімфоїдні вузлики. В свою чергу, лімфоїдні вузлики поділялися на первинні та вторинні. У вторинних лімфоїдних вузликах спостерігали добре виражену мантійну зону та гермінативні центри. Дослідили закономірності росту показників їх площ (середнє значення, медіана та інтерквартильний розкид) та їх кореляцію. Найбільш інтенсивний ріст показників площі мантійної зони та гермінативного центру спостерігали з 30-ї по 60-ту добу. Встановили відносну площу мантійної зони та гермінативного центру у складі вторинного лімфоїдного вузлика. Її значення протягом дослідного періоду майже не змінювалось.

Ключові слова: лімфоїдна тканина; лімфоїдні вузлики; гермінативні центри; мантійна зона; інтерквартильний розмах

Introduction

The immune system is one of the most important systems of body homeostasis, which determines the immune status and adaptability. It is presented by a functional set of lymphoid organs and lymphoid cells of the body providing proliferation, differentiation and migration of immunocompetent elements (Owen et al., 2013; Callahan & Yates, 2014). In addition, the immune system provides tolerance to the own body cells and prevents adverse effects for its own cells that can occur during protection against pathogens (Marinkovic & Marinkovic, 2020). The most mature component of the immune system is gut-associated lymphoid tissue (GALT). It promotes the maturation of the immune system in the postnatal period of ontogenesis and is involved in providing resistance to

pathogens (Arrazuria et al., 2018; Wang et al., 2020). Its main function like other secondary lymphoid organs is not only protection against microbial agents but also against tumour cells (Eberl, 2007; Mörbe et al., 2021).

The main difference is rabbit gut-associated lymphoid tissue has more developed structure than in other mammals due to the presence of two lymphoid formations: sacculus rotundus and vermiform appendix (Butler et al., 2013; Smith et al., 2013). Together with Peyer's patches sacculus rotundus and vermiform appendix contain an organized component GALT – aggregated lymphoid nodules (Zhai & Lanning 2013; Beyaz et al., 2017). These structures are located in the places of the most probable invasion of pathogens that increases the chance of meeting lymphocytes with the corresponding antigen (Perez-Shibayama et al., 2019). Therefore, Peyer's patches, sacculus rotundus and vermiform appendix are the first to respond to antigens getting through gastro-intestinal tract with food and water as well as inform the body about their nature (Khomych & Fedorenko, 2020). In particular, a direct access to intestinal antigens into Peyer's patches is due to their subepithelial location resulting in producing a great amount of IgA (Prados et al., 2021).

Due to stimulation by foreign antigens, structural formation of lymphoid nodules and their functional maturation accelerates in the immune formations (Gomez de Aguero et al., 2016; Stebegg et al., 2018). Central, marginal and subepithelial zones are formed in them (Furukawa et al., 2020). After antigen stimulation GALT lymphocytes turn into effector cells to provide immune response (Khomych & Fedorenko, 2020). The germinal centers, where B-cell of memory and long-lived plasmatic calls secreting antibodies, are formed. Intestinal microbiota including bacteria, fungi, viruses and protozoa affects the germinal centers as well. It causes the production of IgA antibodies involved in the control of intestinal microbial homeostasis (Reboldi & Cyster, 2016; Stebegg et al., 2018). The germinal center B-cells clustering surrounded by the mantle zone with naïve IgD+ B-cells and memory cells on the periphery of the lymph nodule. The main function of B-cell marginal zone is providing a rapid response to pathogens and secretion of natural antibodies (Palm & Kleinau, 2021).

Therefore, the aim of our research was to determine the ratio of functional zones in the secondary lymphoid nodules of Peyer's patches in age aspect in the rabbits of Hyplus breed.

Material and Methods

The study involved Hiplus precocious meat rabbits, divided into 3 age groups of five animals each in 30th-, 60th- and 90th-day-old. Experimental rabbits were clinically healthy, unvaccinated, untreated against ecto- and endoparasites. After slaughter of animals for histological examination, Peyer's patches of the cecum were selected. Selected fragments were fixed in 10% formalin solution or in Buen's solution, and then poured into paraffin, histological sections, which were stained with hematoxylin-eosin and azure-II eosin according to standard methods were made. Microphotographs were taken using a microscope with a Leica DM 1000 camera, morphometric processing of microphotographs was performed using the ImageJ program followed by statistical processing.

The ratio of the mantle zone areas and the germinal centers in the secondary lymphoid nodules was found by the formula:

$$\delta_{1ij} = \frac{Sm^*_{ij}}{Sm^*_{ij}+Sg^*_{ij}},$$

where Sm^*_{ij} is the value of mantle zone area of *i-j* microphotograph, Sg^*_{ij} is the value of the germinal center of i-j microphotograph, $j = \{30, 60, 90\}$.

The mean value $\overline{\delta_{1j}} = \frac{1}{M_j}\sum_{i=1}^{M_j}\delta_{1ij}$ of estimating the ratio of mantle zone area to the secondary nodule on sections *j-I* of age group.

The value $(1\text{-}\overline{\delta_{1j}})$ is estimating the ratio of the germinal center areas to the area of the secondary lymphoid nodule.

Results

Peyer's patch was revealed on the mucous membrane of the caecum on the 30th day of rabbits' development. They had formed the interfollicular region and primary and secondary lymphoid nodules. The mantle and the germinal center were present in the secondary nodules (Fig. 1).

Analyzing the area of the mantle and the germinal center of Peyer's patch of the caecum during postnatal ontogenesis predominant increase of mean values and medians was revealed

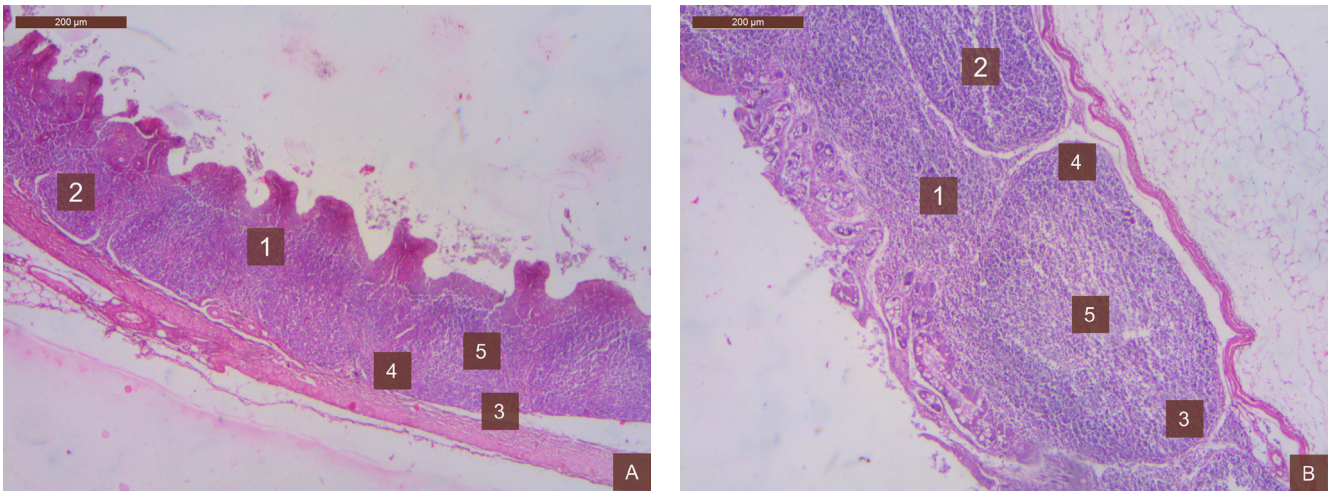

Fig. 1. Peyer's patch of the caecum in rabbits A – 30-days old, B – 90-days old: 1 – the interfollicular region, 2 – the primary lymphoid nodule, 3 – the secondary lymphoid nodule, 4 – the mantle, 5 – the germinal center. Stained with hematoxylin-eosin, ×40

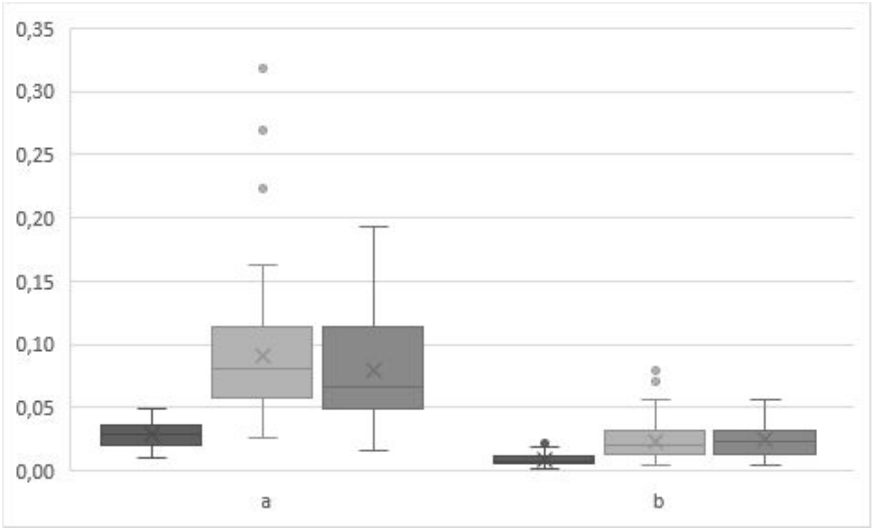

Fig. 2. The area of the functional zones of the secondary lymphoid nodules of Peyer's patch of the caecum: 1 – the mantle area, b – the germinal center area

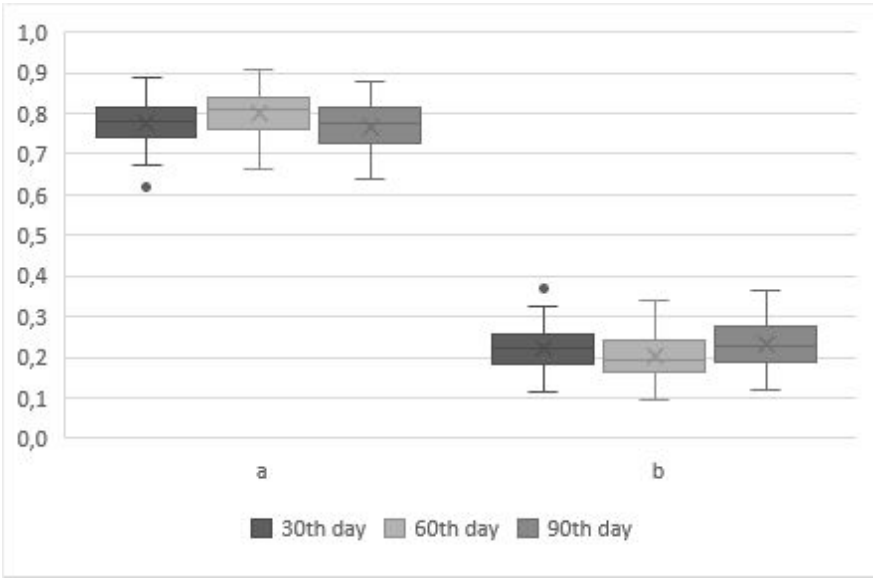

Fig. 3. Relative area of the functional zones of the secondary lymphoid nodules of Peyer's patch of the caecum: a – the mantle area, b – the germinal center area

(Fig. 2). The values of medians and mean values were close. The lowest values of the mantle area and the germinal center were on the 30th day of life. Thus, on the 30th day the mean value and the median of the mantle area were approximately 0.028 mm^2. By the 60th day of life these values of the mantle area (mean and median) 3 times increased and remained almost unchanged until the 90th day.

Increase of interquartile range value has similar pattern. The lowest value of the interquartile range was on the 30th day of life – 0.015. By the 60th day this value 4 times increases and is 0.057. Insignificant increase of the interquartile range occurred by the 90th day of life (0.065).

Similar patterns of increasing the area are characteristic of the germinal centers as well. On the 30th day of life mean values and medians of the area and the germinal center are the lowest – 0.008 mm^2 and 0.007 mm^2 respectively. They 3 times increase by the 60th day and almost do not change by the 90th day (0.024 mm^2 and 0.022 mm^2 respectively).

The ratio of the mantle zone area and the germinal center as components of the secondary lymphoid nodules were analyzed (Fig. 3). It was estimated that on the 30th, the 60th and the 90th day of the development their relative share is almost the same. The relative mantle zone area during experimental period was approximately 80%. The inverse pattern is characteristic of the area of the germinal center, which was 20%. Positive correlation between values of mantle area and germinal center was observed. On the 30th day of life correlation coefficient was 0.7 and on the 60th and the 90th it was 0.8.

Discsussion

The mucosa-associated lymphoid tissue contains main components: follicle-associated apithelium, subepithelial dome regions, B-cell follicles and T-cell interfollicular regions (Özaydın et al., 2018). As soon as pathogen encounters M-cell in follicle-associated epithelium, it is presented to dendritic cells and

macrophages. Subsequently, these cells activate lymphocytes and B-lymphocytes in germinal centers differentiate into IgA-secreting plasma cells (Bonnardel et al., 2015; Panneerselvam, & Budh, 2020).

According to the studies by Pikor et al. (2020) effective response of the germinal center is provided by the movement of B-cells between microenvironment due to interaction of B-cells with reticular cells. It was revealed that even in the primary B-cell nodule specification of reticular cells was determined. While remodeling of follicular dendritic cells of light and dark areas required interlinking connections with B-cell, the output signal from the germinal center when B-cells are kept in the nodule and controlled interaction with nodular T-helpers (Pikor et al., 2020).

Having investigated Peyer's patches in mice Biram et al. (2020) found the necessity of help to T-cells containing signaling lymphocyte activation molecule-associated protein for participating in B-cell selection to the germinal center. In the mice with the lack of this protein plasmatic cells are generated propotional to the size of the germinal center.

In Zhang et al. (2020) research when mice were immunized with sheep erythrocytes decrease of immune response of the germinal centers in the spleen was observed. Decrease of their number and size occurred. No changes in the germinal centers of mesenteric lymph nodes and Peyer's patches were observed. Similar results were noted in mice infected with lymphocytic choriomeningitis virus. There was a skew of the B-cell composition in the dark and light areas of the nodules (Zhang et al., 2020).

Biram et al. (2019) established that Peyer's patches in $Ccr6^{-/-}$ $Aicda^{Cre/+}Rosa26^{Stop\text{-}tdTomato/+}$ mice contained the germinal centers significantly less in number and size than in wild type mice.

In sheep fetuses, according to Özbek & Bayraktaroğlu (2019), precursors of Peyer's patches were found at the 96th day of development, from the 115th to the 119th days the follicle began to expand to the muscular membrane (tunica muscularis). Mature Peyer's patches with the germinate centers were observed on the 147th day of fetuses development as well as on the early postnatal period. In addition, involution of nodules and their expand to the muscular membrane (tunica muscularis) was observed on the early postnatal period (Özbek & Bayraktaroğlu, 2019).

In fetuses of goats rectal patch was detected on the 30th day of development, and on the 5th month of development there was an increase in the size and number of lymphoid nodules and their histological maturity, the presence of a marked germinal center in particular (Indu et al., 2018).

Conclusion

Peyer's patches in rabbits' caecum were detected on the 30th day of life already with present of the primary and the secondary lymphoid nodules and the interfollicular region.

The mantle zone and the germinal center area indices increased almost 3 times from the 30th day to the 60th day of life but from the 60th to the 90th day they remained almost unchanged.

Relative mantle zone area as a component of the secondary lymphoid nodule during experimental period was approximately 80% and the germinal center was 20%. There is a positive correlation between the values of the areas of the mantle zone and the germinal center.

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