

Morphometric Assessment of Functional Immunomorphology of White Rat Spleen in the Age Aspect

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Abstract This article is devoted to the actual problem-the development of the spleen as an organ of the immune system at the stages of postnatal ontogenesis. In an experiment on 45 white mongrel male rats of 5 age groups using histological and morphometric methods followed by statistical analysis, data on the morphometric parameters of the spleen of white rats in postnatal ontogenesis are presented. It was found that significant changes in the structure of the white pulp of the spleen of white rats in the postnatal period occur against the background of involutive processes.

Keywords Rats, Spleen, White pulp, Age-related changes, Morphometric parameters

1. Introduction

The immune system of humans and animals is one of the most sensitive systems of the body, which quickly reacts to external influences [4].

The largest peripheral organ of the immune system is the spleen [1,7].

The rat spleen has a histostructure similar to that of a human organ [10]. The study of structural and functional features of the spleen is an urgent problem, since the spleen, as the largest organ of immunogenesis, is responsible for the effectiveness of cellular and humoral immune response of both innate and acquired immunity [2,6].

The immunocompetent compartment of the spleen parenchyma is represented by its white pulp (BP), where two main B - and T-dependent zones are formed, that is, the areas of localization mainly of B - and T-lymphocytes. These are respectively lymphoid follicles (LF) and periarterial lymphoid couplings (PALM) [1;3].

The study of reactive changes of the spleen, in particular the structural elements of PD, devoted a large number of scientific studies based on the study of this body as a person [3], and various agricultural and laboratory animals [3;5].

Despite the presence of numerous modern studies on the

structure of the spleen under the influence of external factors [4], the morphofunctional changes of lymphoid formations of the spleen in the age aspect remain insufficiently studied. For an accurate representation of the reactions of the lymphoid structures of the spleen on some external stimuli or alien elements, it is necessary to know the morphology lipofilnykh structures of the spleen were normal.

The aim of the work was to study the regularities of the age dynamics of the histological structure of the white pulp of the spleen of white mongrel rats in postnatal ontogenesis from birth to 12 months of age.

2. Materials and Methods

The study was conducted on 45 white male mongrel rats, weighing from 20 to 280 Gy., which were kept in a vivarium with a standard diet, free access to water, the usual mode of purification. The slaughter of rats was carried out under ether anesthesia at the age of newborns, 90, 180, 270 and 360 days from the beginning of the experiment. The rats were divided into five age groups, 9 individuals each: group 1-newborns, group 2 - 90 day rats, group 3-180 day rats, group 4 - 270 day rats, group 5 - 360 day rats. The studies were carried out in compliance with the rules of humane treatment of animals, which are regulated by the "Rules of work with experimental animals" approved by the ethical Committee of the Bukhara state medical Institute. Abu Ali Ibn Sino (No. 18 of 16.01.2018), and were based on the provisions of the Declaration of Helsinki Of the world Medical Association of 1964, supplemented in 1975, 1983, 1989, 1996, 2000, 2002, 2004, 2008, 2013 gg.

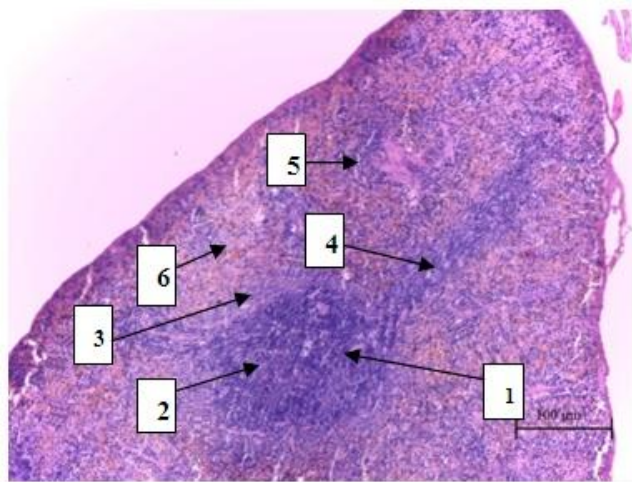
To conduct a morphological study, fragments of the spleen were fixed in 10% formalin solution, carried out through a battery of alcohols and poured into paraffin blocks according to generally accepted methods. Paraffin sections 5-8 μm thick were stained with hematoxylin-eosin. Histological and morphometric studies were performed using a microscope Leica CME, with a digital camera Leica Microsystems CH-9435. The relative area of the white pulp (in%), diameters of lymphoid nodules (in microns), germinal centers (in microns), width of the mantle zone (in microns), marginal zone (in microns) of lymph nodes were determined on the sections of the tear.

The mathematical treatment is produced directly from the total data matrix "Excel 7,0" involvement opportunities "program STTGRAPH 5.1" determined the indicators of the standard deviation and the margin of error.

3. Research Result

In newborn white rats, the spleen outside is covered with a thin connective tissue capsule, from which trabeculae extend deep into the organ. The white pulp of the spleen in newborn white rats is just beginning to form. To differentiate periarterial lymphatic clutches and lymphoid nodules is difficult, the generated nodules is possible to define the mantle and marginal zones.

In histological preparations, the relative area of the white pulp of the spleen of newborn white rats was $17.16 \pm 1.06\%$, the diameter of the lymph nodes was 242.76 ± 15.22 microns. In lymph nodules visually determined: mantle zone, marginal zone, periarterial area. The width of the mantle, marginal and periarterial zones was 35.28 ± 1.89 microns, 64.32 ± 2.07 microns, 44.16 ± 2.46 microns, respectively. Germinal centers of lymph nodes in newborn white rats with histological sections undetected.



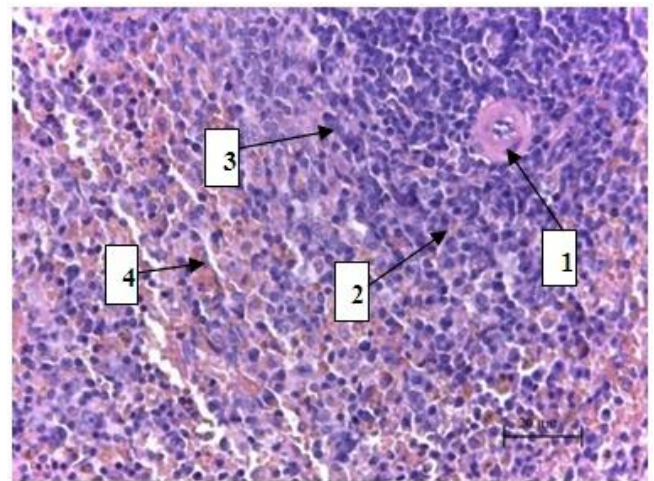
1-lymphatic nodule, 2 - mantle area, 3 - boundary area, 4 - periarterial area, 5 - periarterial lymphoid clutch, 6 - red pulp. Color: hematoxylin-eosin. HC.X100 (90 days of age)

Figure 1. Rat spleen structure

The study of histological preparations of other age groups of the spleen of white rats allowed to establish certain relationships of its structural and functional zones. The spleen of white rats has a well-defined connective tissue capsule and trabeculae containing blood vessels.

The spleen parenchyma of white rats is represented by red and white pulp. The composition of the red pulp includes sinusoidal capillaries and splenic cords. The white pulp consists of numerous lymph nodes and periarterial lymphoid couplings (Fig. 1).

The lymphoid tissue of the spleen, forming a white pulp, on sections is a rounded or elongated gray areas among the red pulp filled with erythrocytes. The white pulp is a cluster of lymphocytes around the eccentrically located arterial canal (Fig.2).



1-Central artery, 2-mantle zone, 3-marginal zone, 4-red pulp Color: hematoxylin-eosin. HC.X100 (90 days of age)

Figure 2. Lymph node of white pulp of rat spleen

In the preparations of the spleen of 3-month-old white rats in comparison with newborn rats, there is an increase in the relative area of the white pulp to $22.2 \pm 1.72\%$, and the diameter of the lymph nodes to 466.05 ± 15.24 microns. The diameter of the germinal centers is 147.8 ± 7.66 microns. The width of the mantle, marginal and periarterial zones was increased to 45.32 ± 1.98 microns, 77.14 ± 3.26 microns, 85.04 ± 2.62 microns, respectively.

Microscopic examination of the spleen of 6-month-old white rats in comparison with newborn rats shows an increase, and in comparison with 3-month-old rats, a decrease in the relative area of the white pulp to $18.54 \pm 0.98\%$, and the diameter of the lymph nodes to 420.96 ± 10.08 microns, respectively. The diameter of germinal centers in comparison with 3-month-old rats decreases to 135.08 ± 2.38 microns. The width of the mantle, marginal and periarterial zones in comparison with the previous age groups increases to 46.56 ± 0.79 microns, 80.72 ± 1.31 microns, 89.42 ± 2.06 microns, respectively.

In the preparations of 9 and 12 month age groups, all indicators of the white pulp of the spleen of white rats gradually decreases. The relative area of the white spleen

pulp of 9 and 12 month old white rats is $16.38 \pm 0.92\%$ and $12.32 \pm 1.08\%$. The diameter of germinative centers in comparison with 3-month-old rats decreases to 414.84 ± 9.14

microns and 407.98 ± 8.18 microns, respectively.

Morphometric parameters of white pulp of spleen of white rats by age aspect are presented in the table.

Table 1. Changes in morphological parameters of white rat pulp in postnatal ontogenesis (M+m, n=45)

Indicators	Groups of white rats according to age aspects				
	newborn	90 day	180 day	270 day	360 day
Area of white pulp of spleen (V %)	17.16 ± 1.06	22.2 ± 1.72	18.54 ± 0.98	16.38 ± 0.92	12.32 ± 1.08
Diameter of lymph nodes (in microns)	242.76 ± 15.22	$466.05 \pm 15.24^{**}$	$420.96 \pm 10.08^{**}$	$414.84 \pm 9.14^{**}$	$407.98 \pm 8.18^{**}$
Diameter of germinative centers (MKM)	-	147.8 ± 7.66	135.08 ± 2.38	127.62 ± 3.16	120.02 ± 5.36
Mantle zone width (MKM)	35.28 ± 1.89	45.32 ± 1.98	46.56 ± 0.79	$44.76 \pm 0.96^{**}$	41.32 ± 1.12
Width of the boundary zone (in microns)	64.32 ± 2.07	77.14 ± 3.26	$80.72 \pm 1.31^{**}$	76.34 ± 2.42	72.52 ± 3.56
Periarterial zone width (in MKM)	44.16 ± 2.46	$85.04 \pm 2.62^{**}$	$89.42 \pm 2.06^{**}$	$84.97 \pm 3.28^{**}$	$79.98 \pm 4.96^{**}$

Note: * mark the valid interval ($p < 0.05$) by compared with previous age

** mark the valid interval ($p < 0.01$) by compared with newborn baby rats

The white pulp of the spleen of 3-month white rats presented a pronounced lymphatic nodules and lymphoid periarterial couplings among lymph nodules is dominated by bundles of germ centers. Have a 6 month old albino rats preobladayut width, mantle, marginal zone and the periarterial over other indicators. In 9 and 12 month-old white rats, invasive changes prevail in lymph nodes and periarterial lymphatic couplings.

4. Discussion of the Data

The immune apparatus of the spleen, according to researchers, has a more complex structure than other peripheral organs of the immune system. In the spleen this unit should include areas of white pulp, namely the periarterial lymphatic vagina that envelops everything Polyarnye artery, lymphatic nodules, and venous sinuses [8].

The percentage of white pulp in newborn white rats is quite low. This is due to the fact that in the first month of life, lymphocytes only populate the pulp of the spleen, lymph nodes are small, it is difficult to distinguish zones in them [9]. According to our data, in the following age groups, the proportion of white pulp increased markedly. The highest values of the white pulp area were observed in the spleen of white rats at 3 months of age, in other age groups the size of the white pulp steadily decreased, confirming the fact of age involution of the organ.

Area of white pulp, the diameter of lymph nodules and the diameter of the germ centers the greatest growth is observed at 3 month of age, the width of the mantle zone, marginal zone width and the width of the periarterial zone the greatest growth observed at 180 days of age.

With age, all studied morphometric parameters gradually decreases and the lowest rate is observed at 12 months of age.

The obtained data on the main morphometric parameters of the white pulp of the spleen of white rats by age aspects correspond to and Supplement the literature data on the structure of the organ in intact animals.

5. Conclusions

1. The relative area of the white pulp of the spleen of newborn white rats was $17.16 \pm 1.06\%$, the largest increase in this indicator is observed in 3 months of age ($22.2 \pm 1.72\%$), and the largest in 12 months of age ($12.32 \pm 1.08\%$). After 3 months of age, there is a decrease in this indicator.

2. The diameter of lymph nodes up to 3 months of age increases by 1.92 times compared to newborn rats. After 3 months of age, this indicator also gradually decreases and at 12 months of age is equal to 407.98 ± 8.18 microns.

3. Germinative centers of lymph nodes in newborn white rats with histological sections undetected. The greatest growth of this indicator is observed in 3 months of age (147.8 ± 7.66 microns), and the highest in 12 months (120.02 ± 5.36 microns).

4. The width of the mantle zone up to 6 months of age increases by 1.32 times compared to the period of newborn. After 6 months of age, this indicator also gradually decreases and at 12 months of age is equal to 41.32 ± 1.12 microns. The width of the marginal and periarterial zone the period from newborn to 6 months of age increases, and from 6 months to 12 months of age decreases slightly.

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