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Morphological Indicators Of The Lymphoid Structures Of The Spleen Of White Rats In Postnatal Ontogenesis In The Dynamics Of Age

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ABSTRACT

The article studies the structural parameters of the spleen of white rats in postnatal ontogenesis in the dynamics of age. It was found that in postnatal ontogenesis, morphological and morphometric parameters of the white pulp of the spleen of white rats undergo significant changes, which is reflected in different age aspects. In newborn white rats, the spleen is functionally immature. At three and six months of age, the spleen has the maximum potential for immunogenesis. At the age of twelve months, the processes of age-related organ involution begin, which are expressed by changes in the structure of the white pulp of the spleen

Keyword: *rats, spleen, white pulp, age-related changes, morphometric parameters.*

1. INTRODUCTION:

The immune system is one of the mechanisms of adaptation of the body, which plays an important role in maintaining its antigenic homeostasis [1].

The interest of morphologists in studying the structure of the spleen associated with the intensive development of immunology is steadily growing [2].

The spleen is a large peripheral organ of immunogenesis, expressing the state of immunity in humans and animals, as well as its structural formations [3].

The spleen is a parenchymal organ represented by a pulp and a connective tissue stroma, which is formed by a capsule, trabeculae, and a reticular framework[4].

The spleen parenchyma consists of two functional zones, red and white pulp, having different structures and functions[5].

Currently, an urgent problem is the study of the lymphoid structures of the spleen, which is responsible for the effectiveness of the cellular and humoral immune response of both innate and acquired immunity [6].

The purpose of the study: to study the morphological and morphometric parameters of the lymphoid structures of the spleen of white mongrel rats in postnatal ontogenetic dynamics of age[7].

2. MATERIALS AND METHODS OF RESEARCH

The study was conducted on 54 white male mongrel rats, weighing from 5 to 280 g., which were kept in standard vivarium conditions. The animals were divided into five age groups: group 1 - newborns (n=10), group 2 - 90 day old rats (n=12), group 3 - 180 day old rats (n=10), group 4- 270 day old rats (n=12), Group 5 - 360 day rats (n=10). All experimental studies on animals were carried out in accordance with the "Rules for carrying out work using experimental animals"[8].

The animals were weighed and removed from the experiment at newborn, 90, 180, 270, 360 days of age by instant decapitation under ether anesthesia[9].

The spleen was removed from the abdominal cavity.The absolute mass of the spleen was determined using torsion weights, its linear dimensions: length, width and thickness using a caliper.The mass index of the spleen was calculated using the formula MI = Spleen *100/M animals, where M is the mass[10].

To conduct a morphological and morphometric study of the study, fragments of the spleen were fixed in a 10% formalin solution, passed through a battery of alcohols and poured into paraffin blocks according to generally accepted methods. Paraffin sections 5-8 microns thick were stained with hematoxylin - eosin.The sections were examined morphometrically,

using an eyepiece micrometer DN-107T/ Model NLCD-307B (Novel, China), the diameters of the peripheral lymphatic couplings, lymph nodes and their germinal centers, the width of the mantle, marginal and periarterial zones, the relative area of the white pulp and connective tissue elements of the spleen and the total area of the section were measured.Measurements were carried out in five fields of view of each histological section. The fields of view were chosen randomly[11].

In order to study the cytoarchitectonics of the lymphoid structures of the spleen, cells were counted using a NOVELModelNLCD-307 microscope, at 10x90 magnification, under oil immersion. The cells were counted using a morphometric grid mounted in the eyepiece (10x) of a microscope[12].

The total number of lymphocytes, the number of large, medium and small lymphocytes per unit area of the slice in PALM, in lymphoid nodules without a breeding center were calculated[13].

Using directly from the general data matrix "Excel 7.0" on a Pentium-IV personal computer, using the capabilities of the program "STTGRAPH 5.1", mathematical processing was performed, the standard deviation and representativeness errors were determined[14]..

3. RESEARCH RESULTS

Outside, the spleen of newborn white rats is covered with a capsule, which consists of thin connective tissue. Trabeculae containing arteries and veins extend from the capsule deep into the organ. In the parenchyma of the spleen, red and white pulps are isolated[15]. On the histological section, the red pulp of the spleen consists of venous sinuses and splenic cords located between them. The white pulp in newborn baby rats is represented by visually indistinctly distinguishable periarterial lymphatic couplings (PALM) and lymphoid nodules (LN). In single primary lymphoid nodules, mantle (MnZ) and marginal zones (MGz) can be determined. At this age, LN still don't tap the germ centers (HZ)[16].

Studies have shown that the weight of newborn animals is in the range from 4.4 g to 5.8 g in average of 5.18±0.15 g. the Absolute mass of a body is 0.02 - 0.04 g, on average – 0,032±0,002 g Mass index is in the range from 0,545% to and 0.674%, on average 0,617±0,014%[17].

The spleen length of newborn baby rats ranges from 5.4 mm to 8.4 mm, on average - 7.2 ± 0.32 mm. The width of the spleen is in the range of 1.4-2.9 mm, on average - 2.12 ± 0.16 mm. The thickness of the spleen varied from 0.8 mm to 1.8 mm, on average - 1.2 ± 0.11 mm[18].

The relative area of the white pulp ranges from 14.2 -20.1%, on average - 17.16 ± 0.64%. The relative area of connective tissue elements varied from 5.2% to 6.5%, on average - 5.94± 0.16% (to the total area of the spleen section). [19]

PALM diameter ranges from 90.2 microns to 109.2 microns, with an average of 100.2±2.05 microns. The diameter of the LN is 218.4- 252.2 microns, on average - 242.76 ± 3.65 microns. In a third of the total number of nodules, mantle and marginal zones can be distinguished.

The width of the mantle zone is 28.4 microns to 38.3 microns, on average 35.28 ± 1.07 microns. The width of the marginal zone ranges from 56.3 microns to 69.7 microns, on average 64.32 ± 1.45 microns. The width of the periarterial zone is 38.6 microns to 48.4 microns, on average 44.16 ± 1.06 microns.

The white pulp is formed mainly from lymphocytes at various stages of maturation. There are small, medium and large lymphocytes.

In three-month-old white rats, the spleen has already been formed.When examining the spleen of 3-month-old intact rats, the following data were obtained:

The mass of animals of 3 months of age ranges from 90 g to 130 g, on average 114.16 ± 3.68g. The absolute mass of the organ is 0.3- 0.7 g, on average 0.52 ± 0.037 g. The mass index ranges from 0.333% to 0.551%, on average 0.455±0.024%.

The length of the spleen ranges from 22.4 to 29.2 mm, on average - 26.78 ± 0.63 mm. The width of the spleen is in the range of 4.6-7.4 mm, on average - 5.94 ± 0.26 mm. The thickness of the spleen varied from 1.8 mm to 3.9 mm, on average - 2.92± 0.19 mm.

In histopreparations of the spleen of 3-month-old intact rats, a clearer separation of the organ parenchyma into red and white pulp was observed.The relative area of the white pulp ranges from 19.8% to 26.2%, on average-22.2 ± 0.59%. The relative area of connective tissue elements varied from 5.0% to 6.1%, on average - 5.52± 0.1% (to the total area of the cut of the spleen)

In the white pulp, periarterial lymphatic couplings (PALM) and lymphoid nodules (LN) can be clearly distinguished. PALM diameter ranges from 122.6 microns to 139.6 microns, with an average of 132.14±1.56 microns. The diameter of the lymph nodes increases by 92% compared to newborn baby rats and ranges from 341.8 microns to 486.05 microns, on average 466.05 ± 13.27 microns. LN can be visually divided into primary and secondary, which percentage ratio is 32% and 68%, respectively. In secondary LN, the formed germinative centers are determined. The diameter of the germinal centers ranges

from 94.6 microns to 167.8 microns, on average 147.8 ± 6.73 microns. The lus are large, often merge. The LN of the white pulp of the spleen mainly has a rounded, oval and elongated shape.

In most cases, the LN zones are clearly distinguishable. The width of the mantle zone ranges from 39.7 microns to 49.45 microns, with an average of 45.32±0.89 microns. The width of the marginal zone ranges from 70.3 microns to 84.7 microns, with an average of 77.14± 1.32 microns. The width of the periarterial zone ranges from 81.9 microns to 89.4 microns, with an average of 85.04 ± 0.69 microns.

It was found that the total number of lymphocytes in the LN without breeding centers is 42-53, on average - 47.3 ± 1.01 cells.Lymphoid nodules without reproduction centers contain (per unit area) small lymphocytes - 30-38, on average -34.0 ± 0.74 cells, medium lymphocytes - 10-12, on average -11.0 ± 0.18 cells, large lymphocytes - 2-3, on average - 2.3 ± 0.1 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white pulp of the spleen is 41-53, on average - 47.2 ± 1.1 cells.Periarterial lymphoid couplings contain (per unit area) small lymphocytes -29-37, on average - 33.0 ± 0.74 cells, medium lymphocytes - 9-11, on average - 10.25 ± 0.18 cells and large lymphocytes -3-4, on average - 3.5 ± 0.1 cells.

The weight of 6-month-old animals ranges from 190 g to 240 g, on average 220.2 ± 5.4 g. The absolute mass of the organ is 0.6- 0.9 g, on average 0.79 ± 0.032 g. The mass index ranges from 0.315% to 0.405%, on average 0.358 ± 0.01%.

The length of the spleen ranges from 26.4mm to 35.7mm, on average - 31.76 ± 1.0mm. The width of the spleen is in the range of 4.9-7.7mm, on average - 6.34 ± 0.03 mm. The thickness of the spleen varied from 2.4 mm to 4.2 mm, on average - 3.12 ± 0.19 mm.

In 6-month-old rats of the control group, the relative area of the white pulp of the spleen ranges from 18.2 to 24.6%, on average -20.54 ± 0.69%.The relative area of connective tissue elements varied from 5.6% to 6.7%, on average - 6.21 ± 0.12% (to the total area of the spleen section).

PALM diameter ranges from 128.2 microns to 141.6 microns, with an average of 136.22±1.55 microns. The diameter of the lymph nodes ranges from 380.8 microns to 477.05 microns, with an average of 420.96 ± 10.44 microns. The percentage ratio of primary and secondary LN is 34% and 66%, respectively. The diameter of the germinal centers ranges from 122.4 microns to 147.7 microns, on average 135.08 ± 2.73 microns. The LN of the white pulp has a rounded, oval and elongated shape.

In micropreparations, it is visually possible to distinguish all the zones of LN. The width of the mantle zone ranges from 40.5 microns to 50.4 microns, with an average of 46.56±1.06 microns. The width of the marginal zone ranges from 74.5 microns to 86.2 microns, on average 80.72 ± 1.26 microns. The width of the periarterial zone ranges from 84.9 microns to 94.7 microns, with an average of 89.42± 1.06 microns.

It was found that the total number of lymphocytes in the LN without breeding centers is 52-61, on average - 57.2 ± 0.97 cells.Lymphoid nodules without reproduction centers contain (per unit area) small lymphocytes - 37-43, on average -41.0 ± 0.65 cells, medium lymphocytes - 12-14, on average -13.0 ± 0.22 cells, large lymphocytes - 3-4, on average -3.2 ± 0.11 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white pulp of the spleen is 53-61, on average - 58.4 ± 0.86 cells.Periarterial lymphoid couplings contain (per unit area) small lymphocytes - 38-43, on average - 41.0 ± 0.54 cells, medium lymphocytes - 11-13, on average - 12.0 ± 0.22 cells and large lymphocytes -5-6, on average - 5.4 ± 0.11 cells.

The mass of 9-month-old laboratory animals ranges from 220 g to 280 g, on average 256.33 ± 5.52g. The absolute mass of the organ is 0.7- 1.0 g, on average 0.84 ± 0.028 g. The mass index ranges from 0.302% to 0.370%, on average 0.327 ± 0.006%.

The length of the spleen ranges from 30.3 mm to 38.4 mm, on average - 34.21 ± 0.74 mm. The width of the spleen ranges from 5.1 to 7.9 mm, on average - 6.52 ± 0.26 mm. The thickness of the spleen varied from 2.5 mm to 4.4 mm, on average - 3.21 ± 0.17 mm.

In 9-month-old rats of the control group, the relative area of the white pulp of the spleen ranges from 18.1% to 22.4%, on average - 20.14 ± 0.39%.The relative area of connective tissue elements varied from 5.7% to 6.8%, on average - 6.33± 0.1% (to the total area of the spleen section).

PALM diameter ranges from 132.2 microns to 142.3 microns, with an average of 137.72±0.93 microns.The diameter of the lymph nodes ranges from 378.7 microns to 447.3 microns, with an average of 414.84±6.31 microns. The percentage ratio of primary and secondary LU is 35% and 65%, respectively. The diameter of the germinal centers ranges from 115.4 microns to 142.8 microns, on average 127.62 ± 2.52 microns. The LN of the white pulp has a rounded, oval and elongated shape.

In most cases, the LN zones are clearly distinguishable. The width of the mantle zone ranges from 38.4 microns to 49.9 microns, with an average of 44.76±1.06 microns.The width of the marginal zone ranges from 70.1 microns to 82.4 microns, with an average of 76.34 ± 1.13 microns. The width of the periarterial zone ranges from 78.7 microns to 92.8 microns, with

an average of 84.97 ± 1.29 microns.

It was found that the total number of lymphocytes in the LN without breeding centers is 48-56, on average - 52.2 ± 0.74 cells.Lymphoid nodules without reproduction centers contain (per unit area) small lymphocytes - 36-41, on average -39.0 ± 0.46 cells, medium lymphocytes - 10-12, on average -11.0 ± 0.18 cells, large lymphocytes - 2-3, on average -2.2 ± 0.1 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white pulp of the spleen is 56-65, on average - 60.3 ± 0.83 cells.Periarterial lymphoid couplings contain (per unit area) small lymphocytes -40-46, on average - 43.0 ± 0.55 cells, medium lymphocytes - 12-14, on average - 13.0 ± 0.18 cells and large lymphocytes -4-5, on average - 4.2 ± 0.1 cells.

The mass of 12-month-old white rats of the control group ranges from 260 to 320 g, on average 282.44 ± 6.48g. The absolute mass of the organ is 0.8 - 1.1 g, on average 0.88 ± 0.03 g. The mass index ranges from 0.288% to 0.354%, on average 0.315 ± 0.007%.

The length of the spleen ranges from 34.4 mm to 42.7 mm, on average - 36.57 ± 0.89 mm. The width of the spleen is in the range of 5.3-8.2 mm, on average - 6.56 ± 0.31 mm. The thickness of the spleen varied from 2.7 mm to 4.6 mm, on average - 3.23 ± 0.20 mm.

In 12-month-old white rats of the control group, the relative area of the white pulp of the spleen ranges from 16.2% to 20.8%, on average -18.54 ± 0.49%. The relative area of connective tissue elements varied from 5.8% to 6.8%, on average - 6.48 ± 0.11% (to the total area of the spleen section).

PALM diameter ranges from 131.4 microns to 142.8 microns, with an average of 136.56±1.23 microns. The diameter of the lymph nodes ranges from 370.7 microns to 437.3 microns on average 407.98 ± 7.19 microns. The percentage ratio of primary and secondary LN is 49% and 51%, respectively. HZ is weakly expressed. The diameter of the germinal centers ranges from 110.2 microns to 132.7 microns, on average 120.02 ± 2.43 microns. The LN of the white pulp has an oval and elongated shape.

In micropreparations, it is visually possible to distinguish all the zones of LU. The width of the mantle zone ranges from 36.4 microns to 47.7 microns, with an average of 41.32±1.22 microns.The width of the marginal zone ranges from 68.4 microns to 76.7 microns, with an average of 72.52± 0.89 microns. The width of the periarterial zone is from 74.8 microns to 84.7 microns, with an average of 79.98±1.06 microns

It was found that the total number of lymphocytes in the LN without breeding centers is 46-53, on average - 50.3 ± 0.75 cells.Lymphoid nodules without reproduction centers contain (per unit area) small lymphocytes - 36-40, on average -38.3 ± 0.43 cells, medium lymphocytes - 9-11, on average -10.2 ± 0.21 cells, large lymphocytes - 1-2, on average -1.8 ± 0.11 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white pulp of the spleen is 53-61, on average - 57.8 ± 0.86 cells.Periarterial lymphoid couplings contain (per unit area) small lymphocytes -39-44, on average - 42.0 ± 0.54 cells, medium lymphocytes - 11-13, on average - 12.2 ± 0.21 cells and large lymphocytes -3 - 4, on average - 3.6 ± 0.11 cells.

The study found that the largest increase in the relative area of the white pulp of the spleen is observed at 3 months of age (22.2 ± 0.59%,), and the largest at 12 months of age (18.54 ± 0.98%.).

The relative area of connective tissue elements in newborn baby rats is 5.94 ± 0.14%, this indicator is the lowest in 3-month-old rats (5.52 ± 0.1%), the highest in 12-month-old rats (6.48 ± 0.1%).

The diameter of the palms of newborn baby rats is 100.2 ± 2.05 microns, a high increase in this indicator is observed at 9 months of age (137.72 ± 0.93 microns), and at 12 months of age decreases and is 136.56 ± 1.23 microns.

The diameter of lymph nodes up to 3 months of age increases by 1.92 times (466.05 ± 13.27 microns). After 3 months of age, this indicator gradually decreases, where at 12 months of age it is equal to 407.98 ± 7.19 microns.Germinative centers of lymph nodes in newborn white rats with histological sections have not been detected, the greatest increase in this indicator is observed at 3 months of age (147.8 ± 6.73 microns), and the largest at 12 months (120.02 ± 2.43 microns).

The greatest increase in the width of the mantle, marginal and periarterial zones is observed at the age of 6 months and is 46.56±1.06 microns, 80.72±1.26 microns and 89.42±1.06 microns, respectively. After 6 months of age, these indicators also gradually decrease, where at 12 months of age they are equal to 41.32 ± 1.22 microns, 72.52± 0.89 microns and 79.98± 1.06 microns, respectively.

The total number of lymphocytes in LN without breeding centers is high in 6–month-olds, and in PALM in 9-month-old rats, which are 57.2±0.97 and 60.3±0.83 cells, respectively, and at 3 months of age these indicators are the lowest and are 47.3±1.01 and 47.2±1.1, respectively.

4. CONCLUSIONS

In postnatal ontogenesis, structural and functional transformations are observed in the spleen of white rats, which is reflected in the process of formation of white pulp. In newborn white rats, the spleen is functionally immature, with hard-to-distinguish zones and single lymphoid nodules formed. At three and six months of age, the spleen has the maximum potential for immunogenesis. At the age of twelve months, the processes of age-related organ involution begin, which are manifested by an increase in the relative area of connective tissue elements, a decrease in the relative area of white pulp, the diameter of PALM, LN, HZ, as well as the width of the mantle, marginal, periarterial zone of the lymph nodes of the spleen

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