The International Journal of Business Management and Technology, Volume 8 Issue 5 September-October 2024
ISSN: 2581-3889

Research Article Open Access

The Mouse Spleen' Morphology in dynamic of the Immune Response to Corpuscular Antigen

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An important aspect of the interaction of pathogens with a macroorganism is the bacteria' resistance to the host innate immunity. Namely against attack from macrophages, killer cells, interferons, acute phase proteins and other factors, the main task of which is the recognition of a foreign antigen and the synthesis of high-affinity antibodies. The highly specific antibodies are the main defense against bacteria. Quite a lot of tests have been developed to assess the host's ability to resist infection. The classic model for studying the mammals immune response to foreign antigens remains the determination of the various mice' strains primary immune response (PRI) to corpuscular antigen, most often sheep red blood cells (SRBCs) [1, 2, 3].

Traditionally, the mice PRI to SRBC study is carried out without preserving the animal life, since the technique includes, first of all, determining the antibody-forming cells (AFCs), rosette-forming cells (RFCs) number in the spleen and bone marrow [6, 7, 11].

We have proposed a method for monitoring the formation of the immune response of experimental animals to a thymus-dependent antigen – SRBC, based on the leukocyte migration index (LMI) in the SRBC lysate presence. This method saves the animal life and is distinguished by the fact that the value of the LMI clearly corresponds with the animal's PIR to SRBC and has dynamics similar to the number of the immune RFCs accumulated in the spleen and the blood serum hemagglutinins' titer level ($\log_2 T$) [4, 5].

In addition, we investigated the nature of the macroorganism immune system' reaction to the *Staphylococcus aureus* autoclaved culture (SAAC) introduction. In particular, it has been shown that leukocytes of intact and immunized with SAAC mice *in vitro* respond to the presence of SAAC ' lysate in the culture medium with an increase in LMI [10].

So, SAAC' mice immunization finishing with the adequate immune response formation. In this regard, we assumed that an SAAC is quite suitable for the corpuscular antigen role.

The purpose of this study was the white nonlinear mice immunized with an autoclaved culture of *Staphylococcus aureus* (SAAC) spleen morphological structure study

I. Material and methods.

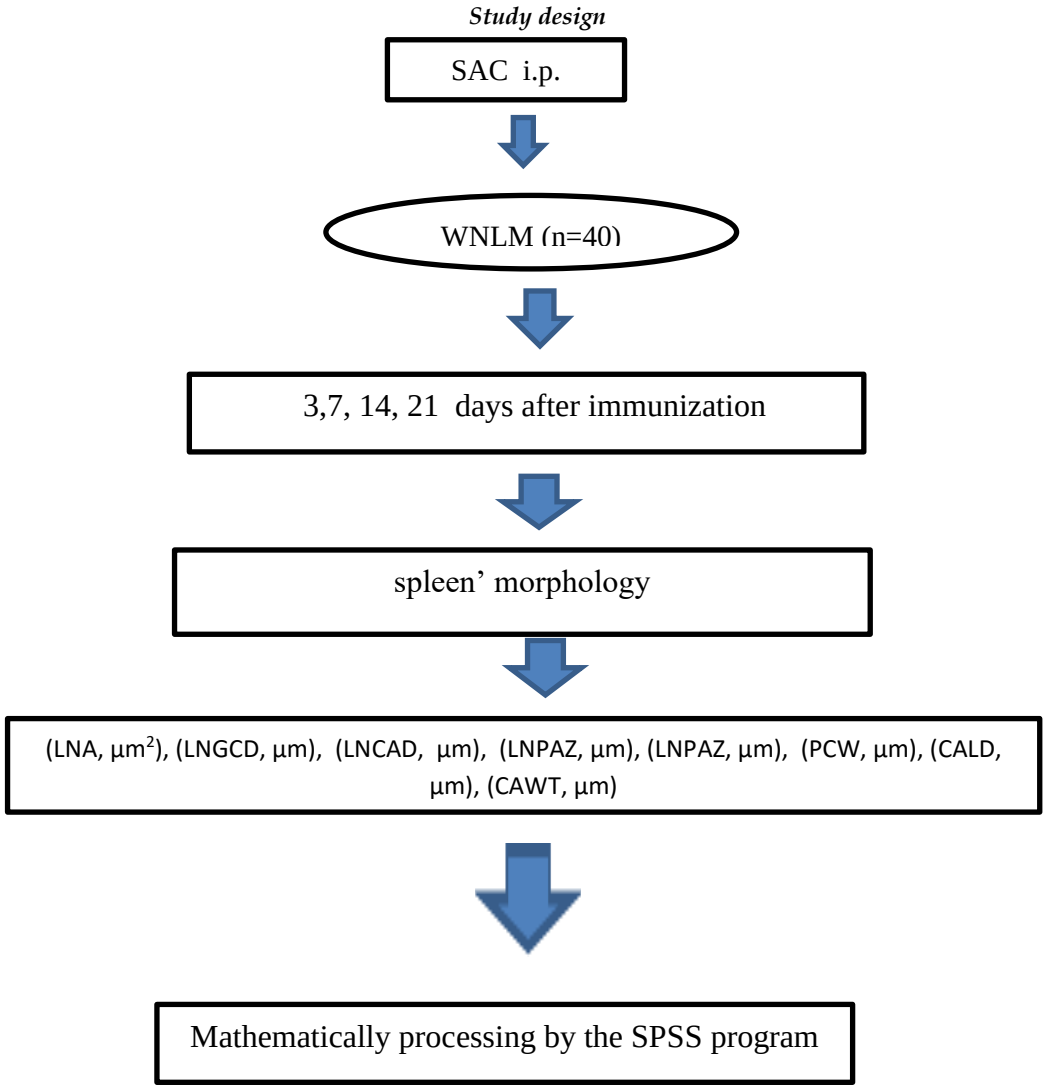
The study used 40 male white non-linear mice (WNM) weighing at least 20 g, obtained from the State Medical University of Turkmenistan named after. M. Garryev' vivarium. The animals were on a standard diet. 2 groups were created, 20 animals each.

In the mice control group 0.5 ml of sterile physiological sodium chloride solution was injected i.p. (group 1 - control), mice in the second group (group II - experimental) were injected i.p. with 0.5 ml of SAAC.

The culture of *Staphylococcus aureus* for research was obtained from the Museum of Microorganism' Strains of the State Medical University named after. M. Garryev Department of Microbiology. A daily culture of bacteria was autoclaved for 45 at 1.5 atm. 1 ml of suspension contained $5x10^9$ according to the turbidity standard.

On days 3, 7, 14 and 21 after immunization, the spleen was removed for morphological studies. Animals were removed from the experiment in accordance with the rules of euthanasia by dislocation of the cervical vertebrae under

ether anesthesia. Organ pieces were fixed in 10% neutral formalin. Further processing of the material was carried out using generally accepted histological methods, paraffin sections with a thickness of 4-5 microns were prepared, and stained with hematoxylin-eosin. Morphometric examination of the preparations was carried out using an Avtandilov grid [8].



The lymphoid nodule area (LNA, μm²), the lymphoid nodule germinal center' diameter (LNGCD, μm), the lymphoid nodule central artery diameter (LNCAD, μm), the lymphoid nodule periarterial zone (the reproductive center) (LNPAZ, μm), width of the periarterial coupling (PCW, μm), the central artery lumen diameter(CALD, μm) and the central artery wall thickness (CAWT, μm).

The obtained digital data were processed mathematically using the SPSS program (USA).

II. Research results.

The study showed that the WNLM spleen morphology corresponds to the generally WBLM accepted spleen parameters - white and red pulp are clearly distinguishable. Lymphatic nodules of white pulp are round in shape.

The periarterial (T-zone) is represented by a lymphocytes' The B-dependent (light) zone lymphocytes dense accumulation is clearly visible. Periarterial sheaths are filled with lymphocytes. The pulp blood vessels are moderately filled with blood. The area of the red pulp was 65.5±4.4; white pulp - 26.7±1.1%; capsules -1.7±0.28%, trabeculae - 5.4±0.7%, respectively. The ratio of the red pulp area to the white pulp area was 2.45. At whole the spleen structure changes nature indicates the humoral immune response formation [9, 12 and 13].

3 days after intraperitoneal administration of ASC to animals, no significant differences were detected in the spleen compared to the control group of animals. . In the red pulp, in places there are minor accumulations of hemosiderin grains. Lymphatic nodules of the white pulp are in most cases round in shape and of the same size. The central arteries are moderately filled with blood.

The T- and B-zones are clearly distinguishable, the marginal zone is pronounced, and the periarterial sheaths are moderately filled with lymphocytes. The red pulp contains minor accumulations of hemosiderin grains and a significant number of macrophages. The area of the red pulp was 71.9±4.5; white pulp – 21,3±2.1%; capsules -2.1±0.28%, trabeculae - 4.9±0.7% respectively. The ratio of the red pulp area to the white pulp area was 3,3.

On the 7th day after immunization, the spleen is hyperemic, venous and sinusoidal hemostasis and rarefaction of lymphatic follicles are observed. The follicles contours are blurred, the follicles are irregular in shape, the follicles sizes are different, the boundaries are unclear. Germinal centers are not detected in all follicles.

The central arteries are moderately filled with blood, and diffuse accumulations of hemosiderin in the red pulp significantly exceed their number compared to 3 days after immunization. The large number of macrophages is also noteworthy. There are a large number of lymphoblasts in the field of view, many lymphocytes with mitotic figures and plasma cells. The area of the red pulp was 78.1±3.8%, white pulp -15.2±1.1%, capsule - 1.3±0.07%, trabeculae - 5.7±0.1% ($p<0$ .05 compared to day 3-d after immunization, in all cases).

The spleen structure nature of changes indicates the formation of a humoral immune response [14].

On the 14th day after intraperitoneal injection of ASC, the lymphatic spleen follicles are even more sparse, their shape and size vary significantly. The follicles shape is irregular, often elongated. The functional zones of lymphoid follicles are poorly distinguishable. The white pulp contains many lymphocytes with mitotic figures. There are many dystrophically changed cells in the germinal zone.

The boundaries of white and red pulp are blurred. There are many destructively altered cells in the periarterial zone and lymphatic sheaths. Trabecular and pulpal vessels are anemic. The red pulp contains many macrophages filled with hemosiderin grains. The spleen area structures are as follows: red pulp - 81.3±8.3% ($p>0.05$), white pulp -13.3±2.7%, capsule - 1.5±5.56%, 4 .9±0.04% ($p<0.05$ in all cases.

It is known that hyperemia of the red pulp and its predominance over the white in bacterial antigen administered mice indicate the formation of a humoral immune response to the same bacterial antigen [15], in the cases of immunocorrection [16] , postradiation status of animals [2] there are the same changes [16]. Consequently, the results of our studies indicate that on 14-t day mice ASC intraperitoneal administrated produces a full-fledged immune response.

On the 21st day, proliferation of the stromal structures of the spleen appears, which indicates regression of the pulpal lymphoid tissue and weakening of its function. In the white pulp there are accumulations of small lymphocytes. The red pulp is sparse. The border between white and red pulp is more distinct. The area of the spleen structures is as follows: red pulp – 74,9±4.4; white pulp - 21.4±1.1%; capsules - 1.7±0.28%, trabeculae - 5.4±0.7%, respectively. The red/white pulp ratio become 3,5.

On the diagram has shown the meaning of the red/white pulp ratio in immune response dynamic's (fig.1).

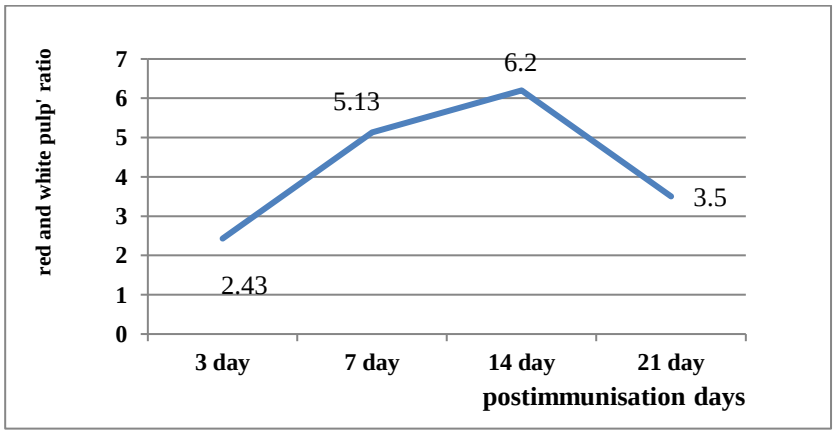


Fig. 1. The red /white pulp ratio in immune response dynamic's

It is clearly seen that the curve of the red/white ratio corresponds to that of the primary immune response of experimental animals to a thymus-dependent antigen. That is, SAAC' mice immunization finishing with the adequate immune response formation. In this regard, we assumed that an SAAC is quite suitable for the corpuscular antigen role.

At a whole, the obtained data showed that the predominance of red pulp over white is observed already on the 7th day of the post immunization. If in mice of the control group the ratio of the red pulp area to the white pulp area is 2.45, then on the 7th day it is already 5.2. (Fig. 1). On the 14th day (at the peak of the immune response) there are clear signs of activation of the red pulp, and the ratio of the areas of the red and white pulp increases significantly to 6.2. Consequently, mice develop a full-fledged primary immune response to SAAC by day 14. On day 21, there is a clear tendency to restore the cellular composition of the white pulp. The ratio of red and white pulp is reduced to 3.5.

The white pulp cytosis increasing indicates the completion of the process of formation of the immune response [11, 17, 18, 19 and 20]. A progressive decrease in the level of red and increasing of the white pulp in the WNM with intraperitoneal administration of SAAC indicates not only the full-fledged mice immune response, but the possibility of SAAC using as the corpuscular antigen.

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