CYTOLOGICAL AND IMMUNOLOGICAL REACTION OF LYMPHOID ORGANS TO INTRAVENOUS INJECTION OF ENTEROVIRAL ANTIGEN

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The number of papers devoted to the role of the lymphoid organs in the production of antibodies to corpuscular and soluble antigens has increased in recent years. Using the method of transplanting lymphoid tissue from an immunized donor to an immunologically tolerant recipient a number of authors [7, 14, 15, 18, 19] were able to conclusively demonstrate the role of the lymph nodes and spleen in the production of antibodies. Similar results were obtained upon transplantation of lymphoid tissue incubated with antigen [16, 17, 20-22]. In addition, the importance of plasmatic cells in the synthesis of antibodies was exposed [3-6, 8, 9, 11].

The participation of lymphoid organs in the production of anti-viral immunity has been less studied. The role of the regional lymph nodes during intranasal [10], intramuscular [2, 13] and subcutaneous [1] injection of antigen has been shown using the model of the influenza virus. During a study of the dynamics of the cytomorphological changes in the regional and contralateral lymph nodes it was established [1] that they are similar in nature to the changes observed upon injection of corpuscular antigen.

We attempted to study the immunological and cytomorphological reaction of the spleen and lymph nodes in response to intravenous injection of a viral antigen.

EXPERIMENTAL METHOD

Poliomyelitis virus type 1 strain Mahoney Li Sc Cinci in the form of an undiluted cultural virus-containing liquid with a titer of $10^{7.5}$ $TTsD_{50}$ ml 50/ml was used as the antigen for immunization.

The experiments were carried out on rabbits weighing 2.5-3 kg.

Antigen in a dose of $10^{8.5}$ $TTsD_{50}$ ml (sic) was injected into the ear vein twice with an interval of 24 days. On the 3rd, 5th, 8th, 11th, 14th, 16th, 18th, 22nd and 14th day after the first and on the 3rd, 5th, 7th, 10th, 12th, 14th, 16th, 18th and 22nd day after the second immunization three rabbits we killed by complete bleeding. 3-3.5 liters of physiological solution was passed under pressure through the greater circulation. This achieved careful washing of the blood from the organs. The right popliteal lymph node and the spleen were removed sterily for study.

The titer of the virus neutralizing antibodies was determined in the blood serum and homogenates of the lymph node and spleen. To prepare the homogenates, the spleen and lymph node were pulverized in a glass homogenizer and made up to a 20% suspension by weight, adding physiological solution containing antibiotics. The suspension obtained was centrifuged in the cold for 30 min at 3000 rpm, the supernatant liquid drawn off and kept at 4° until the time of the study.

Titration of the virus neutralizing antibodies was carried out in tubes with a single-layer culture of monkey kidney tissue against 300 TTsD of type 1 poliomyelitis virus. In the determination of the antibody titer of the lymphoid organs the initial dilution of the homogenates was taken into consideration.

To study the dynamics of the cytomorphological changes of the spleen and lymph node, impression smears were prepared which were stained with methyl-green-pyronine. Calculation of the plasmatic cells was carried out by the

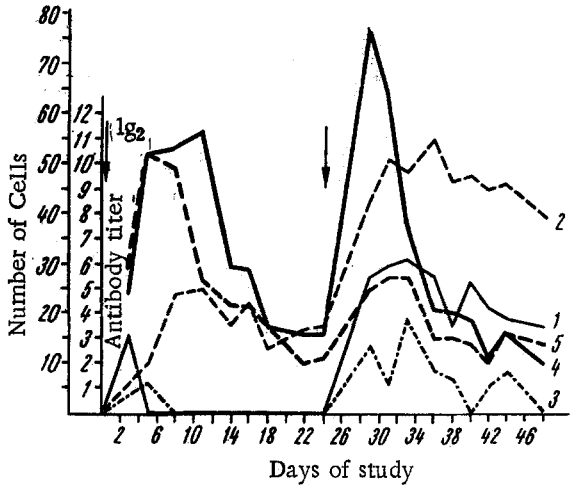

Fig. 1. Dynamics of antibody accumulation in lymphoid organs and blood in comparison with the plasmocytic reactions in the same organs. 1) Spleen antibody titer 2) blood serum antibody titer; 3) lymph node antibody titer; 4) plasmatic cells in the spleen; 5) plasmatic cells in the lymph node.

method described by Gurvich and Shumakova in which the number of hemocytoblasts,* immature, and mature cells were counted separately.

EXPERIMENTAL RESULTS

We first calculated the number of plasmatic cells in the spleen and popliteal lymph node of normal rabbits from the same group of rabbits on which the later study was carried out. In an examination of six normal rabbits the average number of plasmatic cells in the spleen was 14 ± 3.26, in the popliteal lymph node, 12.5 ± 3.08. In the future we considered this data as the norm.

As the studies showed cytological changes in the spleen and lymph node showed up in a significant increase in the number of plasmatic cells; this increase generally occurred at the expense of hemocytoblasts and immature forms. The increase in the number of hemocytoblasts and immature plasmatic cells could be observed on the third day after immunization, while the number of cells in the lymph node obtained on this day was maximal, whereas in the spleen it continued to increase up to the eleventh day. Beginning with the eighth day in the lymph node and the eleventh day in the spleen the number of plasmatic cells decreased, sharply at first and then gradually, and attained the norm at the end of the third week. Simultaneously with accumulation of plasmatic cells (on the third day after immunization) antibody in the spleen in a titer of 3.3 lg_2 could be observed in all the animals. Up until the second immunization we were not able to find antibodies in the spleen. They were found in the lymph node of only one rabbit on the fifth day after immunization. Apparently the antibody titer in the spleen and lymph node during the first immunization with enteroviral antigen lies beyond the limits of the sensitivity of this method of determination, like the neutralization reaction.

Antibodies in the blood serum could also be observed on the third day after immunization although their titer was lower than in the spleen (1.1 lg_2). On subsequent days the antibody titer in the blood serum continued to increase gradually, attaining a maximum on the eleventh day (5 lg_2); then it began to decrease very slowly.

The dynamics of the cytological changes in the spleen and lymph node in comparison with the antibody titer in the blood and homogenates of these organs are presented in Fig. 1.

Comparison of the cytological shifts in the lymphoid organs with the antibody level in the blood shows that accumulation of plasmatic cells and antibody formation in the spleen precedes the accumulation of antibodies in the blood.

The second immunization was characterized by rapid and large cytological and immunological shifts in the lymphoid organs and by a significant increase in the blood antibody level.

In response to a repeated injection of antigen, cytological changes in the spleen and lymph node were primarily noted. They showed up particularly in the spleen where the plasmocytic reaction to the second immunization turned out to be significantly higher than the reaction to the first (Fig. 2, a). In the lymph node the cytological shifts showed up more weakly (Fig. 2, b).

In both cases the overall increase in the number of plasmatic cells generally occurred at the expense of the hemocytoblasts and immature plasmatic cells. The number of mature plasmatic cells changed very slightly. The maximum number of plasmatic cells was noted on the fifth day after which it decreased significantly.

*According to a suggestion of a committee of experts in 1959 the term "hemocytoblast" which included such terms as lymphoblast, and "transitory cells," Fagraeus [12], was introduced.

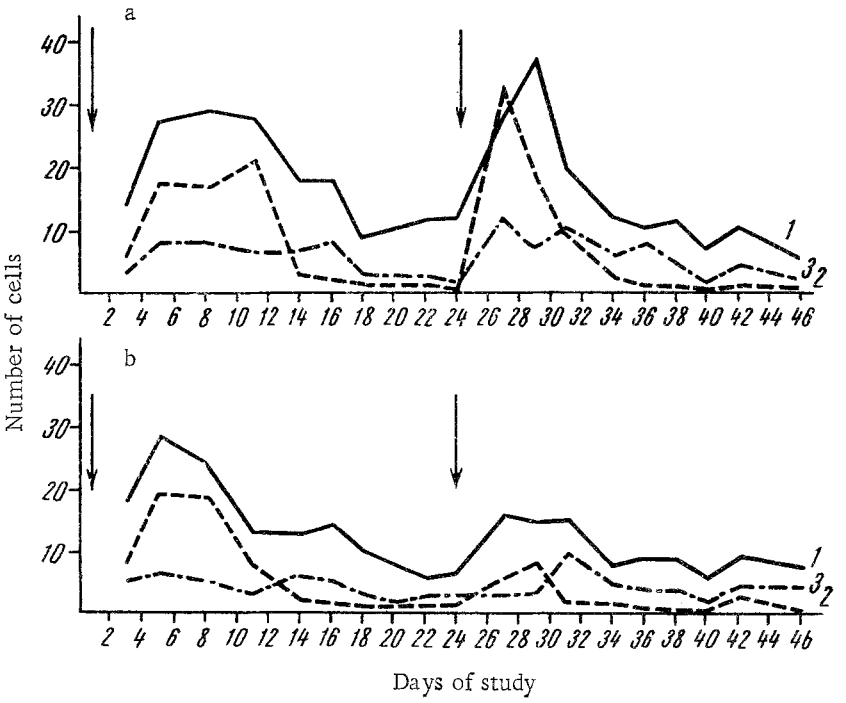

Fig. 2. Dynamics of cytological shifts in the lymphoid organs. a) In the spleen; b) in the lymph node; 1) immature plasmatic cells; 2) hemocytobasts; 3) mature plasmatic cells.

Following the cytological shifts, accumulation of antibodies in the spleen and lymph node was observed. The fact that the cytological and immunological shifts in the spleen took place at a higher level than in the lymph node drew attention. The curves of the amount of antibodies in the blood, spleen and lymph node repeated each other with the only differences that the first occurred at a higher level than the second, while the second exceeded the third. The blood antibody titers (8-10 lg_2) exceeded the spleen antibody titers by ten or more times (3.5-6.3 lg_2) which in its turn exceeded the level in the lymph node by 4-5 times (1.0-3.8 lg_2). Only a comparison of the periods of maximum accumulation of antibodies in the lymphoid organs and blood shows that the reaction of the lymphoid organs precedes the highest concentration of antibodies in the blood.

Thus, it may be assumed that upon intravenous injection of viral antigen all the lymphoid organs, and, primarily, the spleen react. This shows up in an increase in the number of plasmatic cells and an accumulation of antibodies. The increase in the number of plasmatic cells in the lymphoid organs precedes the increase of the antibody titer in them, which in its turn somewhat exceeds blood antibody accumulation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**
