THE ROLE OF LYMPHOID ORGANS IN THE FORMATION OF ANTITOXIC IMMUNITY

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In recent years the participation of the lymph nodes and spleen in the production of antibodies has been demonstrated in diverse immunological forms. However, it is not known which lymphoid tissue is involved, or when it engages in the process of immunogenesis.

Therefore, it seemed of interest to us to elucidate the degree of participation of the various lymphoid organs in the systemic immunologic response to injection of an anatoxin, depending upon the character of the injected preparation (crude or depot) and on the immunization scheme.

In connection with this, we followed the suggestion of P. F. Zdrodovskii and undertook a detailed study of the antitoxin content in the blood and in a series of the lymphoid organs (spleen, popliteal, iliac, axillary, subscapular, cervical and mesenteric lymph nodes). Simultaneously, these organs were investigated cytologically. The lymph nodes were taken from both the same and opposite sides relative to the antigen injection.

EXPERIMENTAL METHOD

The experiments were carried out on rabbits, 1.5-2 kg in weight. In all cases the antigen was injected subcutaneously, into the lower portion of the right posterior extremity. The antitoxin in the lymph nodes, spleen and blood was determined dynamically, on the 8th, 10th, 15th, 25th, 30th, 40th, and 60th days following the initial injection of anatoxin, and on the 3rd, 5th, 10th, and 15th day after revaccination. As the antigen we used diphtheria and tetanus anatoxins.

In the first series of experiments we used 36 rabbits for the initial immunization – 18 in each group. Crude anatoxin was injected into the animals of the first group, in a dose of 20 Lf. The same amount of a precipitated preparation (with 1% alum) was administered to the animals of the second group. At each time interval we sacrificed 3 rabbits by exsanguination following the method described in Report I.

In the second series of experiments, performed on 42 rabbits, we studied antitoxin formation under revaccination conditions. For this purpose we performed a second injection of the same dose (20 Lf) of crude anatoxin 3 months after the initial immunization.

EXPERIMENTAL RESULTS

The investigation showed that the initial injection of antigen is accompanied by a latent period, during which it is not possible to demonstrate antitoxin in the lymphoid organs or the blood. Only after the 8-10th day

(with injection of tetanus anatoxin) or 12-14th day (with injection of diphtheria anatoxin) does there begin a weak production of antitoxin in the regional nodes. At approximately the same time or a little later the antitoxin also appears in the blood. The same results were obtained when the rabbits were injected with the precipitated anatoxin.

Regardless of the character of the preparation injected (crude or depot) the remaining lymph nodes and Spleen do not manifest antitoxin accumulation.

Cellular shifts were seen on the side of the anatoxin injection. An increase in the number of plasmoblasts occurred in the regional node at early intervals following the antigen administration. This increase reached its maximum on the 5th day; on the 10th day the cellular reaction subsided. Cellular reaction in the distant nodes and spleen was very weak.

Thus, the initial injection of antigen is accompanied by very weak production of antitoxin in the regional nodes. Apparently at this time the cellular reorganization of the organs is just forming, for the subsequent provision of antitoxin synthesis.

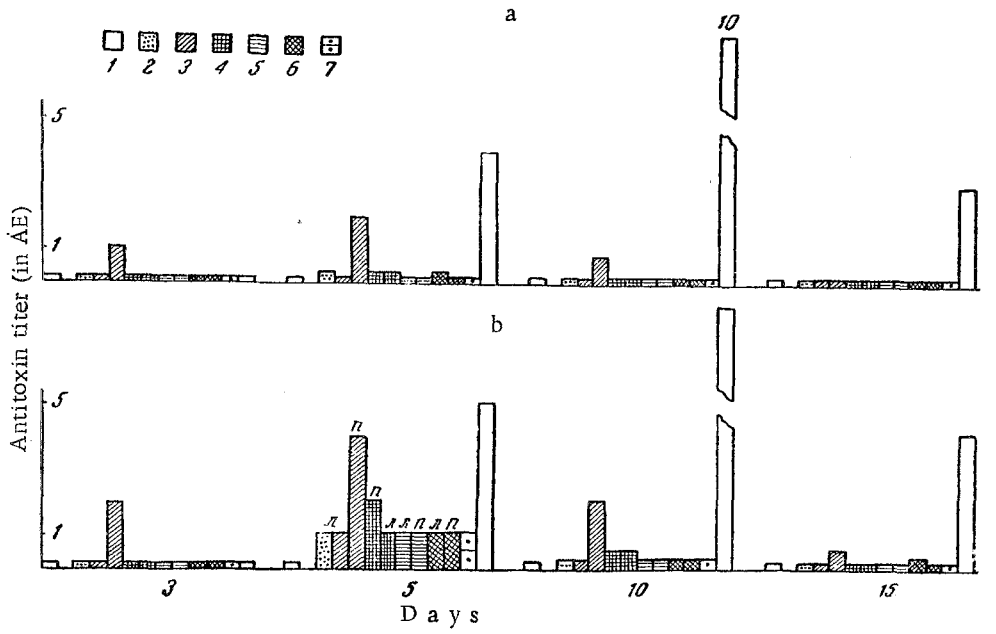

Fig. 1. Content of antitoxin in the blood and lymphoid organs of rabbits following a repeat injection of diphtheria anatoxin. a) Initial and repeat immunization with crude anatoxin; b) initial immunization with adsorbed anatoxin, repeat with crude anatoxin.

The results of antitoxin titration for the lymphoid organs and blood following the second injection of antigen are presented in Fig. 1,a and b.

Regardless of the previous treatment of the animal, the repeat injection of crude anatoxin was accompanied by an early (after 48 hours) appearance of antitoxin in the regional lymph node; the titer here reached its maximum on the 5th to 6th day, with a sharp decline in the subsequent days of observation. The fall in the antitoxin titer for the regional node coincided with a rise in the antitoxin level in the serum. Even by the 5th to 6th day after the anatoxin injection the antitoxin titer in the serum exceeded that in the regional node by many times.

The remaining lymph nodes and the spleen, throughout the entire extent of the investigation, took only a negligible part in the formation of antitoxin. The titer in homogenates of these organs ranged from 0.005 to 0.03 AE. We observed the same relationship in association with the initial immunization using treated anatoxin (alum precipitated and adsorbed). The only difference was in a more energetic formation of antitoxin in

regional node and a transient participation (on the 5th day) of the remaining lymphoid organs in this process (see Fig. 1,b).

The dynamics of the antitoxin formation in the lymphoid organs was the same with revaccination of the rabbits using the precipitated anatoxin. The only difference consisted of a more intense and prolonged (15 days) production of antitoxin by the regional node, and a brief participation of the distant lymphoid organs in this process. However, the titer of antitoxin in these organs was many times lower than in the regional node.

Data on the prolongation of antibody production are also found in the work of Ehrich and co-workers [6], using dysentery vaccine in oil for the immunization. The fall in the agglutinin titer, in this case, began on the 14th day following injection of the vaccine.

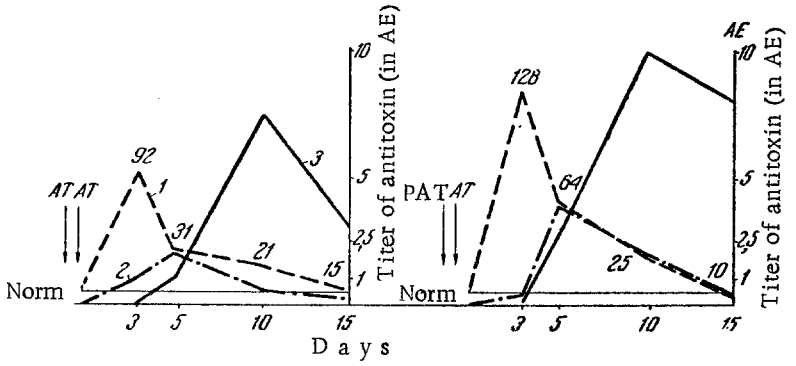

Fig. 2. Cytological shifts in the lymphoid organs. – · – · – plasmoblasts; –.–. titer of antitoxin in the blood; AT – injection of the crude anatoxin; PAT – injection of the precipitated anatoxin.

Thus, use of the treated anatoxin, both for initial immunization as well as for revaccination, caused a generalized type of reaction in the lymphoid organs, i.e., the anatoxin behaved along the lines of a corpuscular antigen, as has been shown in the gertnerovskaya model by G. A. Gurvich and G. V. Shumakova [4].

The dynamics of the cytological changes in the regional node as compared with the antitoxin are presented in Fig. 2.

It is apparent from Fig. 2 that the intensity of the cytological and serological changes depends on the character of the initial immunization. A comparison of the cytological alterations with the curve of the antitoxin formation shows that an accumulation of young forms of the plasma cell series precedes the appearance of antitoxin in the regional node and in the blood.

The majority of investigators [1, 3, 6, 8] have indicated that the period of maximum antibody accumulation in the regional node precedes the cellular reaction. A detailed study of the cellular changes in the lymphoid organs was carried out by a number of authors [2, 8, 9, 10, 11, 12, 16].

Analyzing the responding reaction of the organism to the repeat injection of antigen, we see that the pattern of antitoxin formation does not depend on the character of the initial treatment of the animal, i.e., the regional node engages in the production of antitoxin first. With repeat injection of precipitated anatoxin the difference is seen in a more energetic and prolonged formation of the antitoxin by the regional node and by a brief participation of the remaining lymphoid organs in this process.

We use the term "participation" because the development of antitoxin occurs as a result of active function of the distant lymphoid organs, and not through passive accumulation of the antitoxin in these regions. In the latter case the amount of antitoxin in the lymph nodes and spleen would rise in proportion to increases in the antitoxin level within the circulating blood.

To support the difference we observed in the character of the lymphoid organs' immunologic response to the administration of the different preparations, we set up a supplementary series of experiments.

For the initial immunization the rabbits were injected simultaneously, but separately, with two antigens, administered into the same paw: alum-treated diphtheria anatoxin and crude tetanus. After 10 months these animals were revaccinated with a suspension of the two antigens, without depot formation.

With simultaneous reactivation of both anatoxins the lymphoid tissue reacted with an energetic production of antitoxin to the diphtheria anatoxin and a weak production against the tetanus. The pattern of participation of the lymphoid organs remained as before, i.e., the regional node remained the active producer of antitoxin.

When the structure of the experiment was reversed these lymphoid organs responded with a high production of antitoxin to the tetanus anatoxin and a weak response to the diphtheria.

On the basis of the experiments, carried out on 110 rabbits, we have postulated that the necessary level of antitoxin in the blood is essentially provided by the functioning of the regional node.

This hypothesis is strengthened, in our opinion, by the experiments with extirpation of the regional node. In animals initially immunized with tetanus anatoxin certain organs were extirpated 48 hours after the injection of anatoxin. This series of experiments was carried out on 38 rabbits: in 10 the regional lymph node was removed in 8 – the contralateral node, in 10 the antigen depot was taken out, and the last 10 served as the control (without operative intervention).

The investigation showed that without operative intervention during the period of immunogenesis there was no decrease in the accumulation of antitoxin in the blood. The titer of antitoxin on the 30th day reached 0.1-0.25 AE; at the same time, with extirpation of the regional node 48 hours after the injection of anatoxin there was a sharp decrease (from 10-20 times) in the production of antitoxin (the antitoxin titer was < 0.01 AE).

Comparable results were obtained by Stavitsky [13, 14, 15] and L. N. Fontalin [5], under conditions of both initial immunization and revaccination as well.

Thus, the experiments we carried out on a large number of animals disclosed a significant difference in the reaction of the lymphoid organs depending on the character of the preparation used for the immunization.

Study of the antitoxin formation showed that initial injection of the antigen (crude or depot) is accompanied by a weak production of antitoxin in the regional node, and complete absence of immunologic activity in the distant lymph nodes and spleen.

The immunologic activity of the regional node increases sharply with repeat injection of the antigen. In this case the immunologic reactivity of the lymphoid organs is directly proportional to the intensity of the initial immunization. This appears especially distinctly with the use of preparations that differ in their dispersing property. Thus, crude anatoxin causes a high revaccination effect, limited to the regional node. The introduction of the same antigen to animals initially immunized with depot anatoxin is accompanied by intense synthesis of antitoxin by the regional node and brief participation of the distant lymph nodes and spleen in this process. Finally, the same antigen, injected with 1% alum, causes an overflow type of reaction, with participation of the distant lymph nodes and the spleen. However, in the homogenates of the regional node the content of antitoxin is always higher than in the other lymphoid organs.

On the basis of the experiments carried out we have postulated that the necessary level of antitoxin in the blood is essentially provided by the functioning of the regional node. This hypothesis is strengthened by the experiments with extirpation of the regional node, the latter leading to a sharp decrease in the production of antitoxin.

We were able to show that the possibility exists of controlling the processes of antitoxin formation under revaccination conditions. One can limit the extent of this process to the bounds of the regional node by the injection of crude anatoxin, or expand the process throughout the entire lymphoid system by the use of a depot preparation.

SUMMARY

Experiments carried out on 110 rabbits were devoted to the comparative study of the relationship between immunological reactivity of lymphoid organs and the character of the antigen administered (crude and adsorbed) as well as the immunization scheme. Toxoid was injected subcutaneously into the inferior part of the posterior

extremity. Antitoxin was determined in dynamics in the regional and distant lymph nodes, as well as in the spleen and blood. As shown by the investigations, primary injection of the antigen , irrespective of its character, is accompanied by a weak regional lymph node antitoxin production and complete absence of antitoxin accumulation in the distant lymphoid organs.

With repeated antigen administration the immunological activity of the regional lymph node showed a marked rise. Immunological reactivity of lymphoid organs is directly proportional to the intensity of the primary immunization. Crude toxoid provokes a high revaccination effect limited to the regional lymph node. Administration of the same antigen to the animals which received adsorbed toxoid in primary immunization is accompanied by an intense antitoxin synthesis by the regional lymph node with a brief involvement into this process of distant lymph nodes and spleen. The same antigen administered with a 1% alum causes a diffuse type of reaction.

LITERATURE CITED

1. Z. M. Volkova, A. P. Gindin, and E. V. Vlasova, in the book: Associated Prophylactic Preparations Against Wound Infections (Moscow, 1959) vol. 1, p. 19.
2. A. P. Gindin and Kh. K. Forshter, in the book: Histochemical Methods in Normal and Pathological Morphology (1958) p. 205.
3. S. I. Ginzburg-Kalinina, V. N. Kartasheva, and E. F. Vakarina, Theses from the Reports on the Conference: "Questions in General Immunology " (Moscow, 1959) p. 51.
4. G. A. Gurvich and G. V. Shumakova, Byull. Eksp. Biol. i Med. No. 10, p. 95 (1957).
5. L. N. Fontalin, These from the Reports of the Conference: "Questions in General Immunology" (Moscow, 1959) p. 45.
6. W. E. Ehrich and J. H. Harris, J. exp. Med. 76, 335 (1942).
7. W. E. Ehrich, et al., J. exp. Med. 82, 343 (1945).
8. A. Fagraeus, Acta med. scand. 204 (1948).
9. C. McNeil, Am. J. Path. 24, 1271 (1948).
10. C. McNeil, J. Immunol. 65, 359 (1950).
11. G. Osterlind, Acta path. microbiol. scand. 34 (1938).
12. N. Ringertz and C. A. Adamson, Acta path. microbiol. 86 (1950).
13. A. B. Stavitsky, Fed. Proc. 10, 420 (1951).
14. A. B. Stavitsky, Fed. Proc. 11, 482 (1952).
15. A. B. Stavitsky, J. infect. Dis. 94, 306 (1954).
16. R. W. Wissler, et al., J. cell. comp. Physiol. 50, 1, 265 (1958).

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