PLASMACYTIC REACTIONS AND IMMUNOLOGICAL LAWS

COMMUNICATION I*

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The question as to the site of formation of antibodies, in spite of the many years of study, is still far from having been solved. At present a widening acceptance is developing of cytological alterations which accompany the processes of antibody production and which are denoted the "plasmacytic reaction". Associated with this has arisen the supposition that the plasma cells form the antibodies. Indeed, certain workers have observed directly the production of antibodies by plasmacytes. Thus, Fagraeus in 1948 demonstrated an accumulation of agglutinins in spleen cultures, very richly endowed with plasma cells, while in 1955 Coons, Leduc and Connolly uncovered by means of fluorescence techniques the presence of antitoxin in these cells. When compared with older concepts as to the site of antibody formation such as the reticuloendothelial and lymphoidal theories, the plasmacytic hypothesis does not express a new thought excluding the two just named. On the contrary, it really is an extension of either one or both of them and represents a finer and more contemporary analysis of the developments taking place in the lymphatic glands and spleen after the introduction of the antigen.

The aim of the present investigation was, first of all, to reproduce this phenomenon during the immunization of animals with various antigens and, in the second place, to proceed to a detailed study of the dynamics of the cytological alterations associated with the production of antibodies in the different stages of the process of immunization.

EXPERIMENTAL METHODS

Rabbits weighing 2 to 2.5 kg were immunized with adsorbed diphtheria anatoxin, the injections being made under the skin of the right shin in a dose of 1 cc this corresponding to 65 L_f. The cytological changes in the lymph glands and spleen as well as the blood antitoxin titers were determined 2, 4, 5, 6, 8, 10, 20 and 30 days after the injection. The second injection of the anatoxin was given on the 30th day while the later reinoculation was performed 6 - 8 months later using the same amount of anatoxin and the same site of injection. The cytological alterations and antitoxin titers were determined 2, 4, 6, 8 and 10 days after the second introduction of the antigen. Two to three rabbits were opened on each of the indicated days. Altogether, 46 immunized and 10 normal rabbits were examined.

The cytological alterations were studied from imprints taken of the right and left popliteal lymph glands and of the spleen. The rabbits were narcotized with urethane and opened and the spleen perfused with physiological saline to free it of blood, as an excess of it is reflected in a poor quality of the imprints. The perfusion

*Within our laboratory we have undertaken recently a review of previously established relationships in regard to formation of immune bodies, cytological studies being done on the lymphoid organs—the site of their formation. This is the first of a planned series of studies.

was performed by introducing the solution into the left ventricle of the heart and continued until the fluid drawn from the inferior vena cava became transparent.

The imprints made were fixed in methyl alcohol and stained with azure eosin, some being additionally colored with methyl green-pyronine. In reviewing the preparations 50 oil immersion fields were examined and the plasma cell series were counted, using the terminology of Fagraeus i.e., they were classified as transitional, immature or mature plasmacytes.

Fifty guinea pigs were immunized with heated Gartner paratyphoid vaccine, the dose being 500 million microbes and the injection being made under the skin of the right paw. They were opened under ether anesthesia. The method of preparing the imprints was the same.

EXPERIMENTAL RESULTS

In normal animals the total number of the indicated cells did not exceed 10-15 for the 50 fields examined. In all the immunized rabbits within 2 to 5 days after the introduction of the antigen there was observed a marked increase in the number of the transitory type cells, i.e., cells measuring 8-12 μ, having a regular rounded form and large, more or less fluffy, nuclei with a surrounding narrow rim of basophilic and pyroninephilic protoplasm. This cell reaction was of a rather ephemeral nature, disappearing by the 8th day. The number of mature plasmatocytes in all the preparations was quite small. The most marked cellular reactions were observed on the side on which the anatoxin was introduced. The opposite popliteal gland gave a reaction analogous in character and synchronous in time but less intense. The spleen gave negligible evidence of changes. The dynamics of the cytological changes in relation to the accumulation within the blood of the antitoxin are shown in Fig. 1.

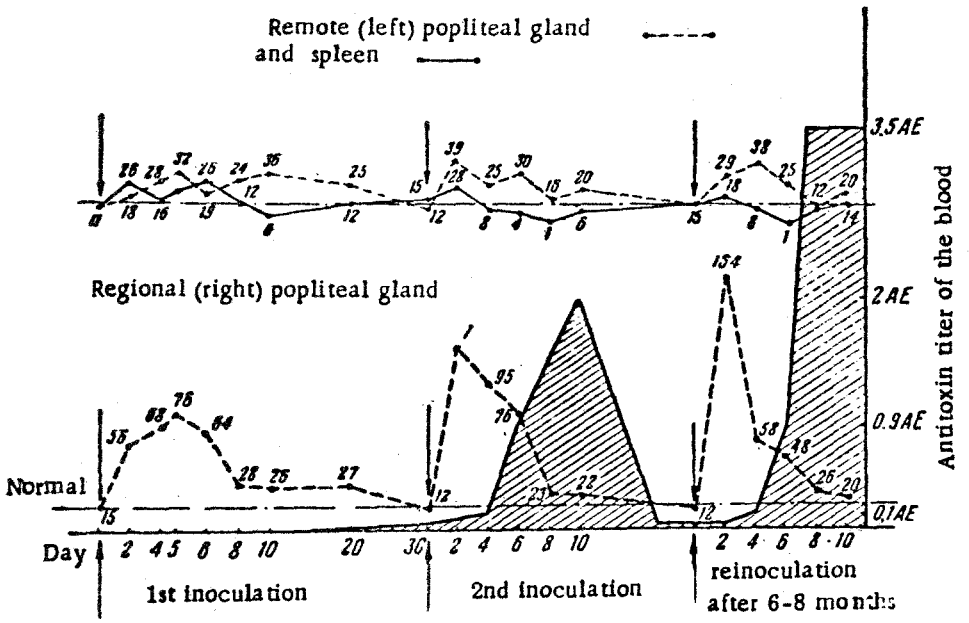

Fig. 1. The plasmacytic reaction and the production of antitoxin during immunization of rabbits with diphtheria anatoxin (average data).

As can be seen from Fig. 1, the cellular response to the second inoculation was more intense than to the first but was most vigorous to the later reinoculation. The antitoxin titer reached maximal values by the 30th day after the primary immunization (0.015 AE); by the 8th to 10th day after the secondary immunization (2AE) and by the 8th day after the later reinoculation (3.5 AE).

The results of the immunization experiments with guinea pigs are shown on Fig. 2.

Within 3 days after the introduction of the antigen, the regional lymph glands showed some increase in the number of plasmacytes, in rabbits as well as in the guinea pigs being at the expense of the less mature transitory

and juvenile cells. After some increase, by the 7th day the intensity of the cell response began to diminish, not reaching base level, however, until the 30th day. In general, the plasmacytic response to the introduction of the corpuscular antigen is characterized by a relatively low intensity and for a considerable length of time the reaction could be still demonstrated.

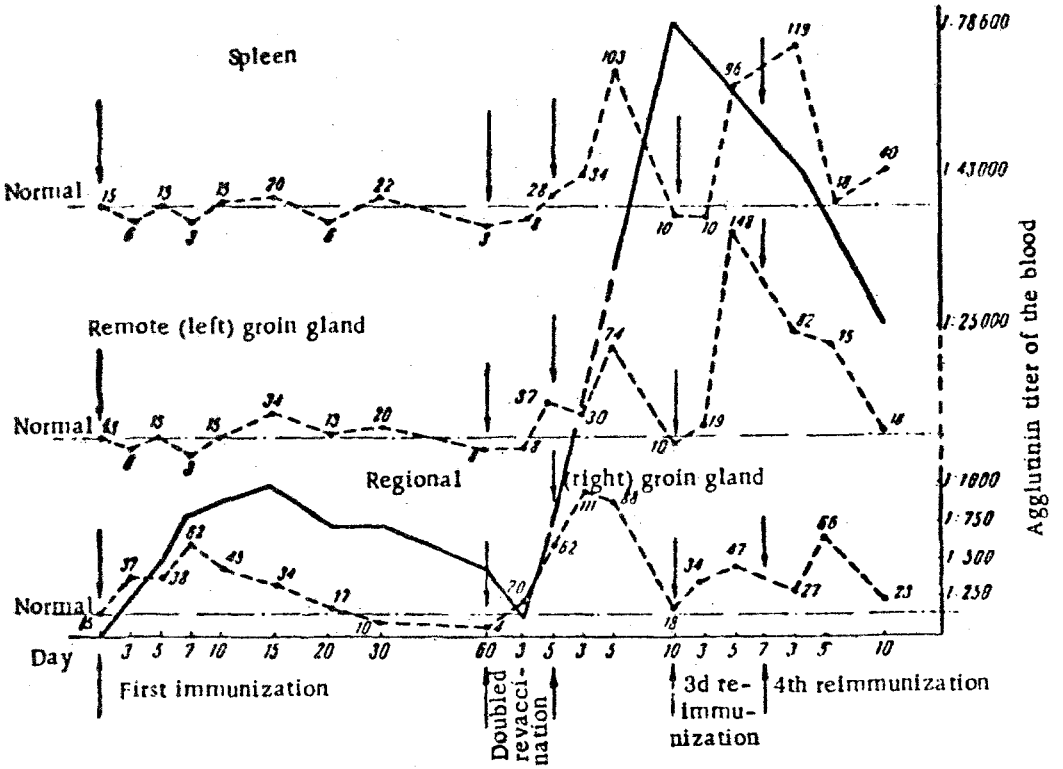

Fig. 2. Plasmacytic reaction and the production of agglutinins during immunization of guinea pigs with paratyphoid vaccine (average data).

The lymph gland in the groin on the opposite side and also the spleen showed some cellular alterations of an analogous type but lesser intensity; in time of appearance, they showed a considerable lag to the regional lymph gland changes.

The plamacytic reaction in the immunological reconstruction of the organism was studied under conditions of "doubling" reimmunization of the guinea pigs two months following the primary inoculation. The lymphatic glands and the spleen were studied before the reinoculation, on the 3rd and 5th days after the first reinoculation and on the 3rd, 5th and 10th days following the second reinoculations. As can be seen in Fig. 2, plasma cells were definitely seen to be increasing from the 5th day after the first reinoculation. By the 3rd day after the second reinoculation the plasmacytic reaction reached a maximum in the regional lymph gland, a maximum being attained in the opposite lymph gland and the spleen by the 5th day. Reinoculation produced more intense cellular responses than did the primary immunization, this result being the same as seen with the anatoxin.

The blood agglutinins increased up to the 15 . day after the primary immunization, reaching an average titer of 1:1000 and then diminishing gradually up to the 60th day. The doubled reinoculation gave a much sharper rise in the titer; the residual reading of 1:460 rose to 1:76,800 by the 10th day.

A careful analysis of the data obtained reveals that the morphological character of the reaction does not correspond completely to the usual descriptions given in the literature. The cytological alterations seen in our experiments both with anatoxin immunization and vaccine immunization demonstrated the preponderance of the early, immature forms described by Fagraeus and other authors as the beginning of the plasmacytic reaction. It is well-known that from the view-point of functional activity these cells are considered to be of the greatest

significance as an indication of the accumulation of the antibodies. However, we did not observe the further development of these forms into the mature plasma cells, i.e., the so-called "maturation" reaction did not take place, instead our experiments showed a regression of the reaction. It thus appears that the stage of mature plasma cells is not essential for the cell reaction being discussed.

The course of the plasmacytic reaction, to a considerable extent, is determined by the character of the antigen. Thus, with the primary immunization with the anatoxin the plasmacytic wave had a definite peak and ended by the 8th day, while with bacterial vaccination the intensity of the reaction was less, the wave rising and falling sharply and lasting some 20 days. The same relationships exist with revaccination. While the second introduction of the anatoxin gave maximal cytologic changes within the day, by the 5th day after introduction of vaccine the cytological alterations were still moderate, the maximal revaccination effect being attained only 5 days after the doubled revaccination. These differences may be due to the circumstance that the anatoxin, being a soluble antigen, acts directly on the lymphoidal tissues while the corpuscular antigen, before becoming a plasmacytic stimulus, has to be phagocytized and be subjected to intracellular splitting. Whatever the reasons, the fact remains there was much less response by the plasma cells to corpuscular antigens than to immunization with anatoxin.

The data obtained show that the general character of the cellular response, regardless of the antigen being used, differs from the first to the secondary challenge and seems to follow definite immunological laws, i.e., the effect of revaccination can be noted. This circumstance seems to be evidence of the close relationship the plasmacytic cytological changes being studied bear to the mechanism of antibody production.

In a search for further evidence of the association of the plasmacytic reaction with the processes of antibody formation, we attempted to determine the state of the lymphoidal elements during plasmacytic alterations occurring during manifistations of immunological inhibition as established in the laboratory of P.F. Zdrodovsky by K.T. Khalyapin, E.F. Vakarina and E.M. Golinevich as well as by A.A. Klimentova and other co-workers. It was supposed that demonstration of the inhibition of the plasmacytic reaction in the lymphoidal tissues, synchronized with immunological inhibition, on the one hand would clarify our understanding of the substrate of immunological inhibition and, on the other hand, would serve as an indirect but still substantial argument in favor of the plasmacytic hypothesis of the production of antibodies. With these goals in mind, the guinea pigs were subjected, after the doubled revaccinations (see Fig. 2) which had produced a very marked immunological response, to two more revaccinations at 7-day intervals. As in the preceding experiments, every 2-3 days three guinea pigs were opened; the spleen and the lymphatic glands of the groin were examined. At the same time, blood was drawn from the animals for serological studies.

The experiments demonstrated that the regional groin lymph gland responds much more weakly to the 3rd and 4th revaccinations, i.e., the plasmacytic reaction shows a state of fatigue. The lymph gland in the groin on the opposite side, which reacted very weakly to the primary vaccination and moderately to the doubled re-immunization, contrariwise, showed a marked response to the third introduction of the antigen and only then entered into a state of inhibition, as the fourth introduction of the antigen did not elicit a response. A very similar curve was obtained for the response of the spleen, the difference being that the marked response came with the fourth revaccination. Thus, it was found that the different lymphoid structures become involved sequentially in the immunological process, each attaining separately a state of maximum activity and then undergoing inhibition. It is quite evident that with such a state of affairs the serological blood picture cannot reflect simultaneously all the immunological processes. Actually, in the entire organism the changes described for the various organs sum up a gradual decrease and the agglutinin titer in the blood gradually falls from 1:78,600 to 1:25,000.

One method of solving the problems presented by this portion of our work would be a separate determination of the antibody titer of each organ, this being the object of our further studies. Making a general survey of our results so far, we conclude that repeated injections of an antigen may lead to inhibition of separate immunologically active organs this being accompanied by a partial decrease of their reactivity, the production of agglutinins to the last revaccination being only a third of the response to the first two revaccinations.

There is still no reason advanced for the fact of the marked lag in the blood antibody titers as compared with the changes occurring in the lymphoidal tissues seen when the rabbits were being immunized with anatoxin. Figure 1 shows that there is a disparity between the two processes expressed by a period of 20-25 days with the

primary immunization and 6-8 days with revaccination. It should be underscored that antibodies begin to increase only some time after the plasmacytic wave has ended. This fact makes it difficult to believe that the plasma cells are the direct agents producing the antibodies.

There may be two explanations for this phenomenon: either the antitoxin is formed by the plasma series cells and then gradually accumulated after some time by the producing organ, or the rise of the plasmacytic cells is not directly associated with antitoxin production but corresponds to some intermediate stage of immunogenesis in a phase preceding the obvious accumulation of the ready antitoxin. Along the indicated lines of thought, we have undertaken special studies in an attempt to determine the amount of the antibodies present in the lymph glands at various time intervals after the introduction of the antigen.

SUMMARY

Rabbits were immunized with diphtheria anatoxin. Guinea pigs were immunized with Gartner paratyphoid vaccine. Immature plasmacytic cells were shown to appear in the regional lymph glands in both instances. After a maximum had been reached, this response waned before antibodies could be demonstrated in the blood.

Repetition of the injections led to an eventual inhibition of this process so that the lymphoid organs ceased to respond with a lymphoid reaction. The regional lymph glands, the distant lymph glands and the spleen did not show a synchronization of the curves of their responses to the primary and the succeeding challenges with the antigen.

The plasmacytic reaction must be closely associated with antibody formation. The precise relationships of the various glands in the process are the object of further studies being undertaken.

LITERATURE CITED

[1] E.F. Vakarina and E.M. Golinevich, Byull. Eksptl. Biol. i Med., XXVII, section 1, 17-19, (1949).

[2] P.F. Zdrodovsky, Zhur. Mikrobiol., Epidemiol. i Immunobiol. No. 9, 3-10, (1950).

[3] P.F. Zdrodovsky, Problem of Reaction in the Study of Infection and Immunity*, (Moscow, 1950) p. 163.

[4] P.F. Zdrodovsky, Zhur. Mikrobiol., Epidemiol. i Immunobiol. No. 7, 3-11, (1952).

[5] P.F. Zdrodovsky, Experimental Immunology and its Immediate Problems*, (Moscow, 1956).

[6] A.A. Klimentova, Zhur. Mikrobiol., Epidemiol. i Immunobiol. No. 5, 28-30, (1950).

[7] A.A. Klimentova, Problems of Infectious Pathology and Immunology*, II, 48-54, (Moscow, 1954).

[8] M.M. Roshkovskaya, Arkhiv Biol. Nauk, XLIX, section 2, 75-83, (1938).

[9] K.T. Khalyapina, Problems of Infectious Pathology and Immunology*, II, 7-23 (Moxcow, 1954).

[10] G.V. Shumakova, Ibid., 55-58.

[11] Coons, Leduc and Connoly, J. Exptl. Med., 102, No 1, 61-71 (1955).

[12] A. Fagraeus, Acta medica Scandinavica, (Stockholm, 1948) Suppl. 204.

*In Russian.