THE PLASMOCYTIC REACTION AND IMMUNOLOGICAL LAWS

COMMUNICATION II. IMMUNOLOGICAL INHIBITION DURING IMMUNIZATION OF RABBITS WITH TETANUS TOXOID

G. V. Shumakova and G. A. Gurvich

From the Gamaleia Institute of Epidemiology and Microbiology of the AMN SSSR (Director – Dr. Med. Sci. S. N. Muromtsev)

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In a previous paper [1] we showed that the characteristic cytological changes accompanying the immunological process and called, not very accurately, the plasmocytic reaction, fully obey the laws of revaccination and immunological inhibition. The applicability of the law of revaccination to the cytological changes was proved for corpuscular and soluble antigens, but the cytological basis of immunological inhibition was studied for corpuscular antigen only. Furthermore it is known that the development of immunological inhibition under the influence of corpuscular antigen differs essentially from the inhibition which arises during the action of soluble antigen (toxoid). In addition, when comparing the periods of development of the cytological changes on the one hand, and the increase in the blood antitoxin level on the other hand, in our first investigations we found a fundamental time lag between the two processes, measuring 20-25 days in primary immunization and 6-8 days in revaccination. Such a marked discrepancy in the timing of these processes merits serious attention in evaluating the role of the so-called "plasmocytic reaction" in immunogenesis, and for this reason the discovery of the causes of this discrepancy requires further investigation.

In view of what has been stated above, under the direction of P. F. Zdrodovskii we undertook a detailed study of the inhibitory process in rabbits caused by the repeated injection of a soluble antigen (toxoid), with separate determinations of antitoxin in the regional and distant lymphatic nodes, the spleen and the blood, and at the same time carrying out a cytological examination of these organs listed.

EXPERIMENTAL METHOD

The experiments were carried out on 52 rabbits which, 6 months previously, had received a single injection of 1 cc of crude tetanus toxoid subcutaneously in the lower part of the hind limb.

The rabbits prepared in this way were subjected to repeated injections of 1 cc of the same antigen in the same area at intervals of 5 days. In a period of 72 days 13 injections were given, with an interval of 10 days after the 7th injection of toxoid.

On alternate days three rabbits were killed by exsanguination, by a method described in the previous paper. After complete removal of blood from the organs to be examined, smears and saline extracts were prepared from them. On the same day blood was taken from the veins of the ear in these rabbits.

Preparation of the extracts. After exsanguination of the animal the right and left lymphatic nodes and the spleen were removed and placed in sterile Petri dishes. Then the above-mentioned organs were weighed on torsion scales (the lymphatic nodes completely, the spleen in part). The weighed tissue was placed in glass homogenizers* shaped like test tubes, and physiological saline was added to the tissue. Maceration of the tissue

* For a detailed account of the technic of homogenization, see Umbreit [2].

TABLE 1

Individual Results of Cell Counts of the Plasmocytic Series in Rabbits in Response to the 1st and 2nd Revaccination

Serial no. of revaccination	Day after revaccination	Rabbit no.	Results of count		
			right lymphatic node	left lymphatic node	spleen
1st revaccination	3rd	1	150/5	19/1	19/8
		2	111/5	17/0	—
		3	138/4	17/0	8/16
		Mean . . .	133/5	18/0	13/12
	5th	4	11/12	9/10	1/18
		5	13/2	14/2	6/18
		6	37/19	11/0	4/16
		Mean . . .	20/11	11/4	4/17
2nd revaccination	3rd	7	164/64	20/0	22/11
		8	137/8	28/0	—
		9	147/4	22/18	7/15
		Mean . .	149/25	23/6	14/13
	5th	10	20/30	8/16	18/68
		11	72/2	16/4	2/36
		12	34/18	6/4	0/13
		Mean . .	42/17	10/8	7/39

Note: The numerator indicates the number of young forms of the plasmocytic series; the denominator – the number of mature plasma cells.

was carried out by means of a pestle which was made to revolve in the tube by an electric motor. The homogeneous mass thus obtained was transferred to sterile tubes, and the homogenate remaining on the walls of the homogenizer tube was twice washed with small quantities of the same solution in order to obtain the required volume. The main dilutions of the organs which were used were extracts diluted 5-10 times, depending on the size of the organ and the period of investigation.

The prepared extracts were placed in the refrigerator, and next day titration of the antitoxin was carried out separately for each organ and the serum. Experience showed that keeping the homogenate for up to 12 days (longer periods of keeping were not tested) does not lower the antitoxin titer. The antitoxin was determined by K. T. Khaliapina's method [3].

Preparation of smears and cytological analysis. The cytological picture of the popliteal lymphatic nodes and the spleen were studied by the smear method. For this purpose the organs were cut up with pointed scissors, and smears were taken from them by careful contact of the cut surface to the glass slide. From 4-6 of these preparations were made, and each smear was taken from a freshly cut surface. The smears were fixed in methyl

alcohol and stained with azure II – eosin. During the examination of the smears, for counting the cells we took in succession all the fields of vision which had a uniform, continuous distribution of cells. In this way, with a magnification of 90 × 7, in each field of vision from 100-200 cells were counted. Lymphoblasts and plasma cells, mature and immature, were counted; the total number of these forms in 50 fields of vision was determined, with meticulous observation of the above conditions of selection of the fields. The results for each period are shown as the average of the cell counts in three rabbits. Examples of the individual figures are shown in Table 1.

As it follows from Table 1, the individual variations in the limits of one period were moderate.

EXPERIMENTAL RESULTS

The investigations established a high content of antitoxin in the regional node, and only on the 3rd day (after 48 hours) after the first revaccination the antitoxin titer was equal to 3 units, reaching a maximum of 13 units on the 5th day. Later, in spite of continuing injections of antigen, the antitoxin level fell, showing from time to time increases in the titers, alternating with new falls, in association with individual variations in the production of antitoxin by the animals.

The same relationships were found in the blood antitoxin curve – the only difference was in the delay in the rise of the antitoxin titer in the first days after injection of antigen. In this case the maximum titer (16.6 antitoxin units) was observed only on the 11th day, after which a gradual fall took place in the antitoxin level.

The lymphatic node of the opposite limb and the spleen hardly participated at all in the production of antitoxin throughout the injection period. The results of this experiment are summarized in Figure 1.

We may mention in passing that weighing of the lymphatic nodes showed a regular increase in the regional nodes to $1\frac{1}{2}$ -2 times that of the lymphatic nodes of the opposite limb.

The results of the cytological investigation of the lymphatic nodes are shown in Figure 2.

As in the previous investigation the main cytological changes consisted of the accumulation of large basophilic cells of a lymphoid type – lymphoblasts, also known as "plasmoblasts" in cases where such an accumulation precedes an accumulation of plasma cells. Since the variations in the content of the latter cells were of quite a different character in our experiments from the variations in the content of young lymphoid forms, these two groups of cells were counted separately. In Figure 2 the variations in the content of each are shown by separate curves.

As shown in Figure 2, each of the first three revaccinations caused a rapid and transient change in the cell composition of the regional lymphatic node, in the form of an accumulation of young lymphoid cells, with a maximum on the 3rd day and a fall on the 5th day. At this period the intensity of each new rise increased, but the depth of the fall gradually diminished. The response to the 4th revaccination was somewhat weaker than that to the 3rd revaccination, and there was no response at all to the 5th revaccination, since in place of the usual increase there was a continued fall in the number of basophilic lymphoblasts. The content of these cells remained at a figure of 65-85, and was practically unchanged after the 6th and 7th revaccinations. After

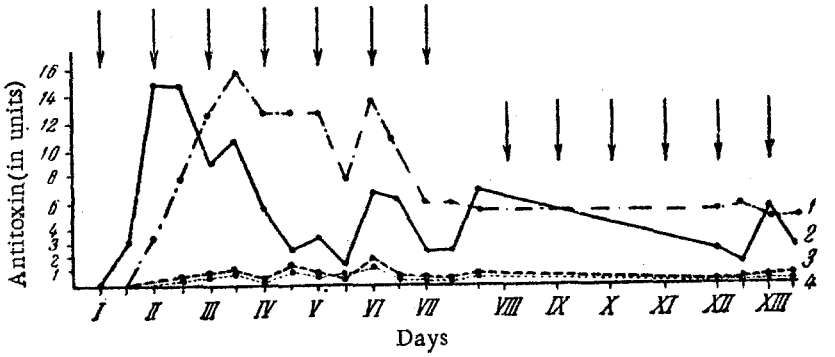

Fig. 1. Accumulation of antitoxin in the blood and organs of the rabbit.
1 – In the blood; 2 – in the right lymphatic node; 3 – the same on the left;
4 – in the spleen. I-XIII – serial numbers of revaccinations.

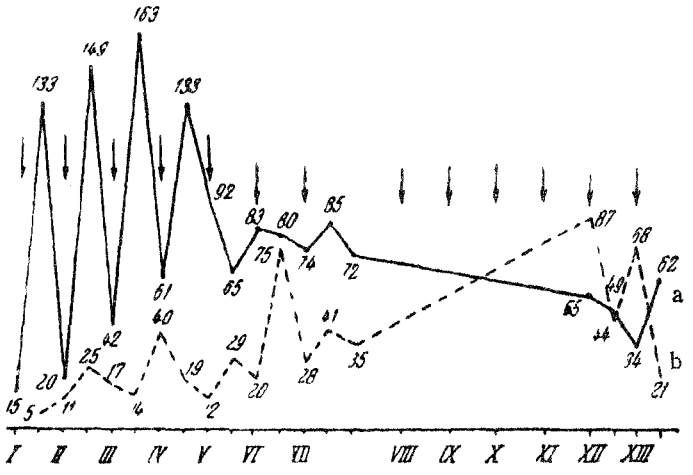

Fig. 2. The trend of the cytological changes after repeated injections of tetanus toxoid (regional node).
a – Immature plasma cells; b – mature plasma cells. I-XIII – serial numbers of revaccinations.

5 more revaccinations, in response to the 12th and 13th injection of antigen, there was no essential change in the content of young basophilic forms.

So far as the adult plasma cells are concerned, their content was low in the first half of the course of revaccinations and rose in the second half of the course. For example, if their average content for the period covering the first 5 revaccinations was 19, in the period between the 6th and 13th revaccinations it was 44.

The character of the quantitative variations in the plasma cells was without that strict regularity which was found in the trend of the young forms (see Fig. 2), and the difference in the individual variations in their content was more significant.

The cytological changes in the distant lymphatic node and also in the spleen throughout the whole course of revaccinations were much less marked. For instance, the average accumulation of basophilic lymphoblasts did not exceed 33 in the lymphatic node on the opposite side, and 20 in the spleen.

After repeated injections of toxoid into prepared rabbits, they develop in their regional lymphatic nodes cytological changes characterized by the accumulation of large cells of lymphoid type with a markedly basophilic protoplasm (lymphoblasts, or plasmoblasts) (see Fig. 3, I, a). Serological investigation of extracts of these nodes shows even at early stages the accumulation of large quantities of antitoxin.

The preferential accumulation of antibodies in the regional lymphatic node was first found by McMaster [8, 9] and Hudack [8], and was confirmed by Ehrich and Harris [4, 5], Harris and Harris [6, 7] and by L. N. Fontalin.*

The frequent repetition of revaccination with toxoid causes development in rabbits of a state of immunological inhibition, which is shown serologically by cessation of the increase in the antitoxin content of the regional node, and by its subsequent fall, and morphologically by absence of the characteristic increase in the number of lymphoblasts in the node.

In reproducing the phenomenon of immunological inhibition we started from the investigations of K. T. Khaliapina, who made a detailed study of this feature in P. F. Zdrodovskii's laboratory.

However, when using a single preparatory inoculation of crude toxoid, we did not achieve such a high level of reactivity as took place in K. T. Khaliapina's experiments, in which two preparatory inoculations were given. The consequence of this variation of K. T. Khaliapina's scheme was a delayed and not-so-sharp fall

* The findings were reported at an extended conference of the Institute of Normal and Pathological Physiology.

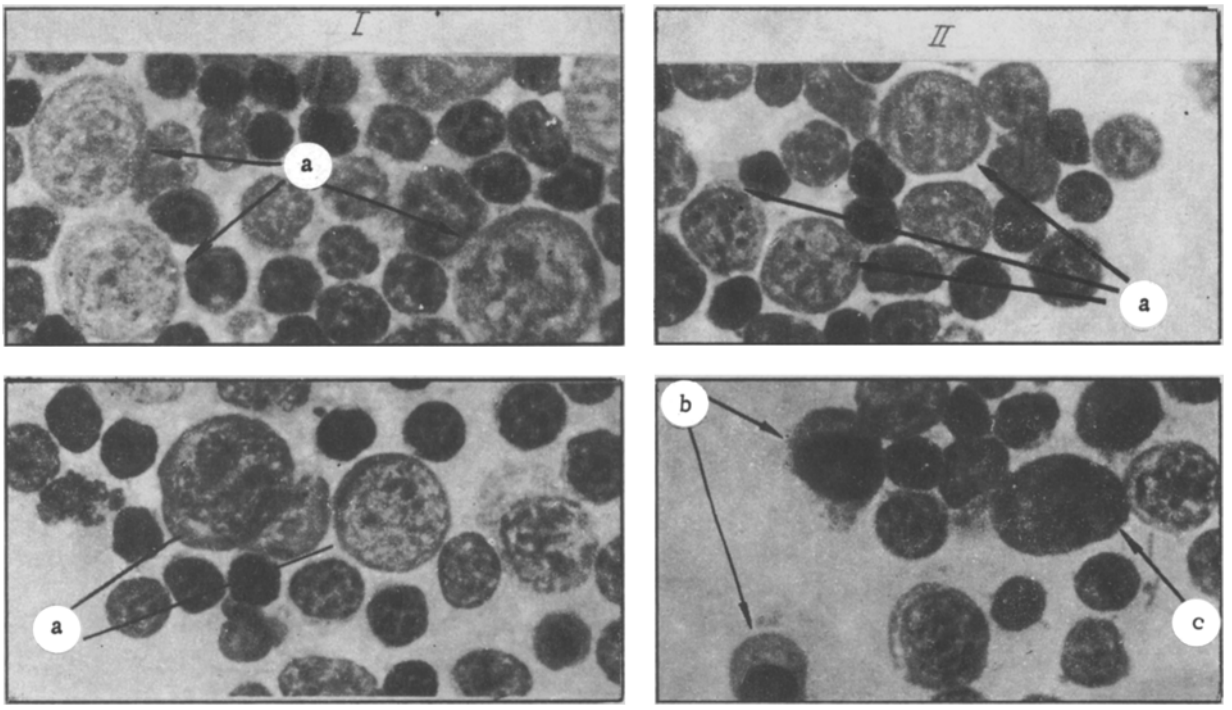

Fig. 3. Smears from the regional lymphatic nodes of rabbits on the 3rd day after the 2nd revaccination (I) and after the 4th revaccination (II).
a – Basophilic lymphoblasts (plasmoblasts); b – immature plasma cells; c – mature plasma cells. Microphotograph, magnification 1000 x.

in the titers of antitoxin in the blood in the inhibitory phase. Even in this less profound inhibitory phase however, the suppression of immunological activity of the node was very significant.

Analysis of the findings reveals the following distinctive features. In the first place, by comparing the times of development of the cytological changes with the trends of the antitoxin level in the regional glands, the complete synchrony of the two processes can be clearly seen. For instance, after the first revaccination (3rd day) a marked lymphoblastic reaction (133) takes place, accompanied by a considerable accumulation of antitoxin (3 units). In this connection the discrepancy previously pointed out between the appearance of the cytological changes in the node and the accumulation of antitoxin in the blood during revaccination is fully explained. The delay in the accumulation of antitoxin in the blood in this case is entirely due to the holding up of antitoxin at the place of its formation, i. e., in the regional node.

In the course of the 2nd and 3rd revaccination the titers of antitoxin in the node are high; the morphological reaction to these revaccinations is very clear. At the time of the 4th revaccination a fall in the antitoxin titers began to be observed. At the same time the first signs appeared of a change in the morphological reaction, shown by the fact that in the interval between revaccinations the morphological picture does not succeed in returning to normal, and the response to the 4th revaccination is weaker than that to the 3rd revaccination. Subsequent revaccinations give a picture of complete inhibition, both morphologically and serologically.

In the second place, the strictly local character of the effect of revaccination is shown with great clarity. In all the rabbits immunized, irrespective of the number of revaccinations, the antitoxin content was highest in the regional node.

The lymphatic node in the opposite limb and the spleen hardly take any part in the production of antitoxin. The morphological observations are in full agreement with the serological findings. The cytological changes, so clear in the regional node, could hardly be detected in the node on the opposite side or in the spleen.

The impression is created that the required level of antitoxin in the blood is ensured by the functioning of one regional lymphatic node alone.

This impression is in agreement with the findings of several other workers. Similar results, for instance, were obtained by Ehrich and Harris [5], and by Harris [6]. By extirpation of the regional node or spleen (depending on the mode of injection of antigen), Stavitsky [13] was able to bring about a sharp fall in the effect of revaccination, from which it could be concluded that the synthesis of antitoxin was local in character. Finally, Oakley [see 11], using transplantates, estimated that 1 g of tissue taken from the site of injection of the antigen produces 800 times more antitoxin than the same weight of tissue taken from any other part of the body.

Thus the production of antitoxin is effected mainly in the regional lymphatic node; more distant nodes play hardly any part in this process, despite 13 successive revaccinations. In this respect the immunological inhibition caused by a soluble antigen differs essentially from the inhibition caused by corpuscular antigens. In the latter case, as was pointed out in the previous investigation, repeated revaccinations led to the successive inclusion in the immunizing process of the more distant lymphatic nodes and the spleen. These distinctive features of the process of inhibition during the action of different antigens is of considerable theoretical interest.

Finally, the last special feature of the process of inhibition during the action of tetanus toxoid consists of the character of the cytological changes in the regional lymphatic nodes. As was pointed out earlier, in primary immunization as in revaccination, the main cytological changes are produced in the field of the young forms of the plasmocytic series – plasmoblasts. The content of adult plasma cells was very inconstant and small. The fall in the number of plasmoblasts, taking place after the rise, was not accompanied by any essential accumulation of plasma cells.

For this reason we have to think either of a reverse development of this reaction or, which is more likely, of its termination in ordinary lymphocytes. However, as the number of revaccinations increases, and the resulting state of inhibition becomes more profound, accumulation of adult plasma cells and all stages of their maturation are observed increasingly often. In these cases the whole series of transitional forms connecting the lymphoblasts to the plasma cell can readily be seen (Fig. 3, II, b, c).

On the other hand, many of the basophilic lymphoblasts, preserving their morphology, did not attain such a large size as we had seen previously. Thus, if after the first revaccinations the diameter of the large basophilic lymphoblasts was 18-20 μ (Fig. 3, I, a), then in the stage of inhibition, the dimensions of the lymphocytes were reduced to 10-12 μ (Fig. 3, II, a).

Thus with the fall in the function of the lymphatic node there was a corresponding reduction in the size of the lymphoblasts and the presence of a considerable number of plasma cells, adult and immature.

SUMMARY

This investigation was devoted to the study of the inhibitory process in rabbits caused by repeated administration of tetanus toxoid. Investigations demonstrated that cytological changes develop in the regional lymphatic nodes of rabbits on repeated injection of the toxoid in sensitized animals. These changes are characterized by the accumulation of large cells of lymphoid type with a basophilic protoplasm (lymphoblasts or plasmoblasts). The serological examination of the extracts of these nodes shows the accumulation of large amounts of the antitoxin even in the early periods.

Numerous repeated revaccination with the toxoid causes a condition of immunological inhibition in rabbits, which is serologically manifested by the cessation of the rise of the antitoxin concentration in the regional nodes and later in its drop.

Morphologically this inhibition is manifested in the absence of the characteristic increase of the lymphoblasts in the lymph nodes. The effect of revaccination was strictly local. The content of antitoxin in the regional lymphatic nodes was low irrespective of the number of revaccinations.

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