THE MORPHOLOGY OF THE ACTIVE AND INHIBITORY PHASES OF IMMUNITY IN GUINEA PIGS IMMUNIZED WITH HEATED PARATYPHOID VACCINE

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Many descriptions have been given [4, 5, 6, 8] of the variations in the immunological reactions of experimental animals receiving repeated antigenic stimuli. It has been shown that under these conditions there is a periodicity in which phases of excitation and depression of antibody formation alternate. Under the influence of the immunization, immediately after the phase of maximal immunity excitation, there follows a phase of depression during which the animals lose the power of producing antibodies, although stimulated by further antigenic stimuli. The refractory phase disappears slowly over a long period.

The reports of this condition do not refer to the morphological changes. The exception is the work of G. A. Gurvich and G. V. Shumakova [1], who describe the immunological changes of the plasmocyte reaction. They showed that during the phase of immunological excitation, there is a considerable increase in the number of transitional forms of plasma cells in a regional lymphatic node, and during the inhibitory phase the regional node responds by a much weaker plasmocyte response, i.e., a condition of fatigue occurs quite frequently.

The object of the present investigation has been to study morphological changes in lymphatic nodes in the spleen of experimental animals, during excitation and inhibition of the immunological processes.

EXPERIMENTAL METHODS

Groups of 25 guinea pigs were immunized with a subcutaneous injection of B. paratyphi A into the left inguinal region, as follows.

Animals of the first group received 1.3 milliard bacterial cells in 1 ml of fluid; the second group were immunized in precisely the same way, but after 26 days they were revaccinated; the third group received 100 million bacterial cells each, in small portions; 13 injections were given at intervals of 2-3 days.

The degree of immunological change was determined from the amount of agglutinins in the blood, which was collected on the days when the antigen was injected. To construct the graph, and also to consider the immunological response, we used mean values obtained by determining the titres of the agglutinins in the blood of 5-10 animals.

For the morphological studies, two guineapigs of the first group were killed on the 2nd, 6th, 12th, 18th and 24th days after immunization, and animals of the second group were killed at the same times after revaccination. In the third group, animals were taken for morphological study after 5, 8, 10, and 13 injections and 15 days after the last injection.

Studies were made of the spleen, of the regional and cervical lymph nodes. The organs were fixed in Zenker-formol, and were embedded in paraffin. Sections 5 μ-thick were stained with azure II-eosin.

Microscopically, there was some increase in the size of the regional lymph nodes and spleen in the animals of the first and second groups. In the third group the regional lymph nodes were enlarged in some of the guinea pigs, and in some they were reduced while in all of them, the spleen had enlarged $1\frac{1}{2}$-2 times.

EXPERIMENTAL RESULTS

A single immunization with heated paratyphoid vaccine caused a marked production of agglutinins, which was maximal on the 11th day (mean titre 1 : 167) and was gradually reduced towards the 27th day. A second injec-

tion of the antigen after an interval of 26 days caused a more intense production of aggulutinins, and the concentration in the blood reached a maximum after 7 days, when the mean titre was 1 : 290; subsequently the level was greatly reduced.

Microscopically, regional morphological changes were found as early as two days after the injection. Large numbers of lymphocytes were rapidly liberated into the sinuses of the cortical and medullary zones, and then, a moderate hyperplasia of the cells of the reticular tissue and of the lymphoid follicles. There were a few plasma cells. There were no noticeable changes in the cervical lymph nodes.

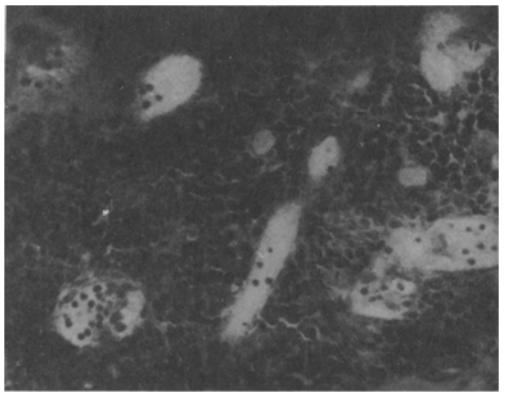

Fig. 1. Regional lymph node of a guinea pig on the 12th day after revaccination. The medullary cords are almost completely filled with plasma cells. Micrograph. Stain azure II-eosin. Magnification: objective 20 ×, ocular 5 ×.

On the sixth day, in the regional and cervical lymph nodes there was a marked hyperplasia of the cells of the reticular tissue, although the change was more marked in the regional lymph nodes. The reticular tissue was noticeably bereft of lymphocytes.

A large number of eosinophils and eosinophilic promyelocytes, metamyelocytes, and plasma cells appeared. Among the latter there was a preponderance of young forms.

On the 12th day, in the regional lymph nodes there was a considerable loss of lymphocytes from the reticular tissue. The cells of this tissue appeared swollen, and tightly packed. Between the cells only occasional lymphocytes could be seen. The medullary cords of the medullary zone contained a large number of young plasma cells. There were also an exceptionally large number of eosinophils, and eosinophilic metamyelocytes, and promyelocytes were specially numerous.

In the cervical lymphatic nodes, the morphological changes were similar, but less marked.

On the 18th day, and still more on the 24th day after immunization, the lymphoid tissue had recovered: between the cortical and medullary zones, and in the medullary cords there were a large number of plasma cells, among which there were mature and occasional degenerate cell forms. There was still a hyperplasia of the cells of the reticular tissue, which remained swollen, and as before there were a large number of eosinophils.

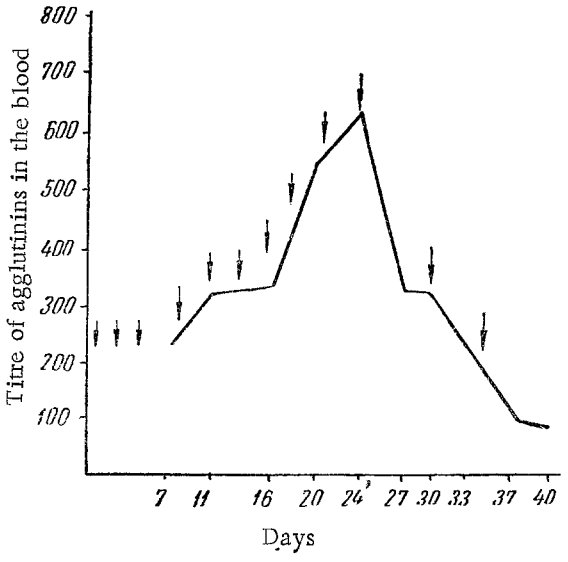

Fig. 2. Level of agglutinins in the blood of guinea pigs following repeated injection of antigen. ↓ Injection of antigen.

After revaccination, on the 6th day, there were dense hemorrhagic infiltrations at the site of the injection, and a great increase in size of the regional lymph nodes and of the spleen. By the 24th day, these changes were only weakly shown.

It was found microscopically that all the changes in the regional and cervical lymph nodes after revaccination showed a still greater accumulation of plasma cells than there had been after the first immunization (Fig. 1).

The response of the spleen was essentially the same as that of the lymph nodes. In the red pulp there was a rapid increase in the number of plasma cells. After revaccination the increase in these cells was still more marked, and practically the whole of the red pulp was thickly filled with them. At the end of the period of observation, i.e., on the 24th day after revaccination, the plasma cells of the spleen were not diffusely scattered, but were gathered in pockets around the trabeculae, beneath the capsule, and along the periphery of the lymphoid follicles. The greater portion of the red pulp contained no plasma cells at this stage.

Results on the degree of immunological reorganization in guinea pigs in response to repeated injection of B. paratyphi A. killed by heating are shown in Fig. 2.

As can be seen from Fig. 2, repeated injection of the antigen caused a gradual increase in the level of agglutinins, which was maximal on the 24th day, when the titre was 1 : 610, and at this time the 10th injection of antigen was given. However, the following three injections caused no noticeable increase in the level of the blood agglutinins, but, on the contrary, a very rapid decrease of their production.

After the 5th injection of the bacterial suspension, no changes could be observed in the regional lymph nodes; i.e., the plasma-cell reaction was the same as it was in animals which had received their first vaccination, and in the revaccinated animals, but it was found that most of the plasma cells were mature, and some of them were degenerating.

After the subsequent injections, in the regional lymph nodes a kind of dedifferentiation of the cells of the reticular tissue became more marked. It showed as a reduction of the basophilia and as a relative reduction of the cytoplasm of the cells; the nuclei were large and vesicular, poor in chromatin, and they had a comparatively small nucleolus. The swollen reticular cells which normally differentiate into plasma cells had become smaller, as had also the transitional cells and the plasmoblasts. In the medullary zone, occassional medullary cords were practically devoid of reticular cells, and consisted of a small number of weakly stained cells and fibrous structures.

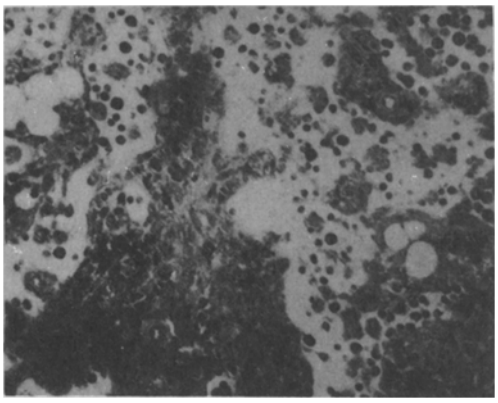

Fig. 3. Regional lymphatic node of a guinea pig treated with 13 injections of vaccine. The medullary cords are almost devoid of plasma cells; part of the medullary cords have atrophied. Micrograph. Stain azure II-eosin. Magnification: objective 20 ×, ocular 5 ×.

The phenomena described were particularly noticeable in the regional lymph nodes of the guinea pigs killed on the 15th day, after the last (13th) injection of antigen (Fig. 3).

In the cervical lymphatic nodes the number of plasma cells was reduced, and of those that were present the majority were mature or degenerating.

The histological structure of the spleen after the 5th injection of antigen showed little external difference from those in the spleen of the animals in the preceding experiments. Just like the red pulp of the spleen of revaccinated animals, that of animals killed after the 5th injection was closely packed with plasma cells.

After the subsequent injections, the number of plasma cells decreased rapidly, and the cells were found chiefly around the trabeculae and the vessels. Most were mature or degenerating. After the 13th injection, there were practically no plasma cells left in the spleen, and there remained only a few clumps of mature and degenerating forms. The cells of the reticular tissue had at this time large, not very clearly defined nuclei which were poor in chromatin. Their cytoplasm stained a weak pale blue color with azure.

From what has been said it can be seen that after immunization, and particularly after revaccination, there was an intense morphological response; it consisted of a rapid reorganization of the cells of reticular tissue both of the lymph nodes (particularly of the regional lymph nodes) and of the spleen, and their transformation into plasma cells. This response corresponds to the active immunological phase, as determined in the present communication by the level of agglutinins in the blood of the injected animals.

When repeated injections of relatively small doses of the same antigen were given, there was a gradual increase of the plasma cell reaction in the same organs, but only up to a certain stage, that of the 5th injection. Subsequently, there was a rapid reduction in the number of plasma cells in the red pulp of the spleen and in the lymph nodes, particularly in the regional lymph nodes. Then mature and degenerating forms of plasma cells preponderated, and there was a general reduction in their number. The medullary cords of the regional lymph nodes became ever more depleted through a considerable reduction in the number of reticular cells.

The reduction in the titre of the agglutinins, as can be seen from Fig. 2 occurred after the 10th injection of antigen.

This reduction almost coincided with the morphological changes which we found.

Recent experiments have shown that antibodies are produced by young plasma cells (Fagreus, 1948). It was just these forms that were present in large numbers in the lymph nodes and spleens of our experimental animals during the phase of active immunological response. Subsequent injection of antigen apparently has the effect that the loss of reticular tissue through differentiation into plasma cells cannot be effected, because the reticular cells themselves have to go through a definite developmental cycle occupying a certain time. It is also possible that reticular cells, like those of other excitable tissues, become refractory or enter into a condition of parabiosis (in the sense used by N. E. Wedensky).

The principal changes observed in the preparations were firstly a considerable reduction in the number of plasma cells, especially of the young forms; secondly a reduction in the number of cells of the reticular tissue, with the results that, for example in the medullary cords of the medullary zone of the lymph nodes, the fibrous structures became clearly visible and contained only a small number of weakly staining reticular cells; thirdly, differentiation of the reticular cells into plasma cells ceased, and no new reticular cells were formed. All these changes accompanied the phenomenon known as the phase of inhibition of the immune reaction.

It must however be supposed that the morphological changes of the inhibitory phase of immunity are compensated, because after a period of rest, the animals once more became able to respond to an antigenic stimulus [2].

The investigation has shown that it is possible to apply morphological methods to the study of certain practical problems of immunity related to vaccination.

SUMMARY

In repeated multiple administrations of heated B. paratyphi A vaccine certain morphological changes took place in the lymph nodes (especially regional) of guinea pigs. These consisted of rapid reduction of the total number of plasmatic cells, especially of the young forms, and of the total number of reticular cells, accompanied by a marked atrophy of reticular tissue.

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