

MORPHOLOGICAL CHANGES IN THE LYMPHATIC GLANDS
AND SPLEEN OF GUINEA PIGS REPEATEDLY INOCULATED
WITH *Rickettsia burneti* (THE MORPHOLOGY OF IMMUNITY)

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From their studies of the conditions and laws of inhibition of immunological activity, P. F. Zdrovskii [1, 2] and workers in his laboratory [4] have concluded that as a result of immunizational overexcitation with an antigenic stimulus of excessive strength, a state of reactive depression develops, which they associate with a varying degree of exhaustion of the reactivity of the recipient. Manifestations of immunological depression are also regularly reproduced, according to their findings, in all cases when one immunizing stimulus is significantly or highly predominant over others, especially when reacting successively with each other. The morphological reactions of these phenomena have not been studied.

The present investigation is an attempt to determine the character of the morphological changes in the lymphatic glands and spleen of guinea pigs during superinfection.

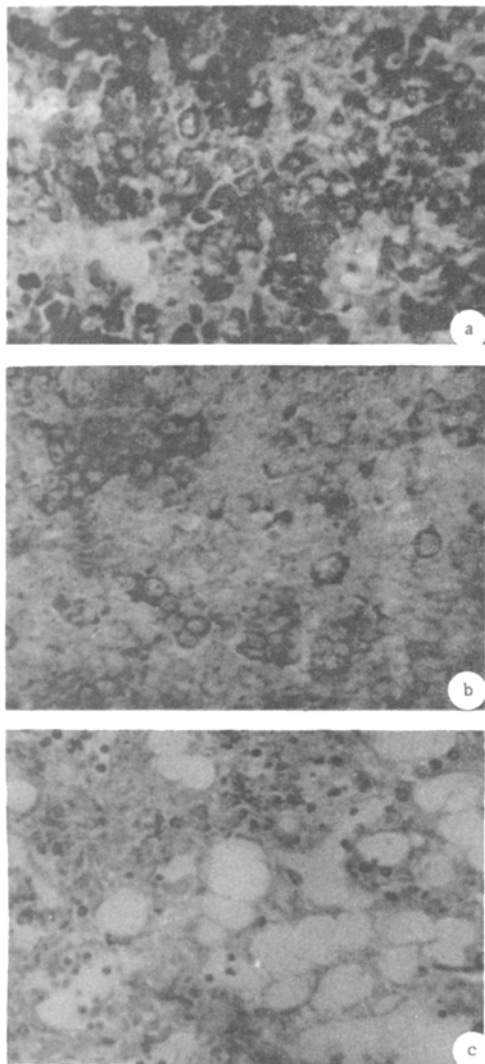
METHOD

Experiments were carried out on 40 guinea pigs weighing 250-300 g. The animals were preliminarily inoculated subcutaneously in the left inguinal region with a strain of *Rickettsia burneti* of low virulence (strain BD-2520, Gudima O. S., 1956) in a dose of 10,000 conventional guinea pig units. Thirty days later the animals were again inoculated subcutaneously in the right inguinal region with a highly virulent strain of *R. burneti* (Italo-Greek strain) in a dose of 100,000 conventional guinea pig units. Guinea pigs were sacrificed (two at a time) for morphological investigation on the 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 20th, 25th, 30th, 40th, 50th, and 60th days after the second inoculation. As a preliminary measure 2 ml of blood was taken from the heart of all the animals for serological investigation. The regional and common mesenteric lymphatic glands and spleen were examined. These organs were fixed in Zenker's formol and embedded in paraffin wax-celloidin. Sections were cut to a thickness of 5 μ and stained with azure II-eosin.

RESULTS

Macroscopically, at the site of the second injection, from the eighth to the 14th days isolated petechiae were seen in the connective tissue layer of the skin and the adjacent subcutaneous connective tissue. The regional lymphatic glands were enlarged, especially during the first six days after the injection; after 14 days they could no longer be seen to be enlarged. The spleen was firm, and slightly enlarged on the first few days; after 10-14 days it was normal in size.

Microscopically, in the lymphatic glands, especially the regional glands, large numbers of transitional cells [3] and plasmoblasts could be seen on the second day after inoculation (see figure, b). These cells were particularly numerous in the cortical zone of the lymphatic glands and in the lymphatic follicles. The cells of the reticular tissue were swollen, and many of them were in a state of mitosis. Another characteristic feature was the



Morphological changes in the organs of the guinea pig after two inoculations with *Rickettsia burnetii*. a) spleen on the second day (large numbers of plasmoblasts and immature plasma cells are seen); b) regional lymphatic gland on the second day (transitional cells and plasmoblasts are seen); c) regional lymphatic gland on the 16th day after the second inoculation (atrophy of the medullary cords of the medullary zone). Photomicrograph. Ocular 20X, objective 20X. Stained with azure II-eosin.

from the spleen of control, uninoculated animals.

At the moment of the second inoculation, the mean titers of complement-fixing antibodies in the serum of the experimental animals was 1:60. After the second inoculation an increase in the antibody titers took place on the sixth day (1:160), reaching its maximum on the 12th day (1:320). A gradual fall in the antibody titers then gradually supervened; on the 40th and 50th days their level was 1:30, and on the 60th day it had risen to 1:120.

presence of numerous mitotic divisions of immature plasma cells. The sinuses of the cortical zone of the lymphatic glands were firmly packed with large lymphocytes and plasmoblasts. Hardly any small lymphocytes were encountered here.

The same picture was observed on the fourth day also, although the mitotic activity of the reticulum cells was appreciably reduced.

On the sixth day, at the sites of accumulation of transitional cells and plasmoblasts, large numbers of adult plasma cells were seen, and these became predominant. The cortical zone of the lymphatic gland was appreciably thinned, the sinuses of the medullary zone were dilated and empty, and the medullary cords were tightly packed with plasma cells.

After the 8th-10th day the number of transitional cells and plasmoblasts fell sharply. The predominant cells became adult plasma cells and their degenerative forms. The number of reticulum cells also fell considerably and hardly any of these cells showed mitotic division.

After the 12th-14th day the comparatively rapid disappearance of all forms of plasma cells from the lymphatic glands was seen, especially in the regional glands. This was observed most clearly in the medullary cords of the medullary zone; on the 16th day all the plasma cells here had disappeared almost completely. As a result the medullary cords were largely atrophic, and appeared as fibrous structures with a comparatively small number of weakly stained reticulum cells (see figure, c). These morphological changes in the lymphatic glands were observed in all successive times of investigation until the 50th day. On the 60th day the medullary cords of the medullary zone of the lymphatic glands were almost normal, i. e., contained many reticulum and plasma cells, but there were comparatively few lymphocytes.

Roughly the same changes were observed in the spleen. On the second day after the second inoculation the entire red pulp of the spleen was diffusely packed with transitional cells and plasmoblasts (see figure, a), among which many mitoses were visible. On the sixth day, however, at the site of the previous accumulations of these cells mainly adult plasma cells were present, arranged more or less in nodes around the trabeculae and lymphoid follicles and under the capsule of the spleen.

On the 12th day comparatively few plasma cells were observed in the red pulp, but the nodal nature of their distribution was even more pronounced. Subsequently, until the 60th day, no changes whatever could be seen. By its morphological structure, the spleen at this time showed no particular difference

As the foregoing account shows, the subcutaneous injection of two large doses of *R. burneti* caused an intensive proliferative reaction of the cells of the reticular tissue of the lymphatic glands and spleen, with the almost complete transformation of these cells into plasma cells. The subsequent pattern of the morphological changes consisted basically of the rapid maturation and disappearance of the plasma cells thus formed. This process passed through a stage of degeneration of the plasma cells. The cessation of the formation of the transitional cells and plasmoblasts, on the one hand, and the accelerated, large-scale maturation of these cells into plasma cells followed by a relatively rapid disappearance of the latter, on the other hand, created the picture of atrophy of the reticular tissue, which was particularly prominent in the lymphatic glands.

As a result of this, the medullary cords of the medullary zone of the lymphatic glands, for example, were almost completely denuded of cells and consisted largely of fibrous structures with a few, extremely weakly stained cells (16th day after inoculation). It was only on the 60th day after inoculation that the medullary cords of the lymphatic glands of the experimental animals showed an arrangement of reticulum cells, capable of undergoing transformation into plasma cells, that was more or less normal in appearance and density. These latter were relatively numerous here.

The curve of titers of complement-fixing antibodies in this experiment followed the same line as the intensity of the plasma-cell reaction, lagging behind it by 6-7 days.

In the author's opinion, the most interesting face in this investigation is the temporary atrophy of the reticular tissue, particularly prominent in the lymphatic glands. This atrophy coincides in time with the lowest level of the titers of complement-fixing antibodies. Manifestations of immunological depression thus take place after repeated antigenic stimulation of considerable strength. The repeated injection of a large dose of *R. burneti* of high virulence caused an intensive but transient proliferation of reticulum cells followed by their subsequent rapid transformation into plasma cells. The impression is thereby created that the stocks of reticulum cells capable of such a transformation were apparently expended. The previous injection of a strain of *Rickettsias* of low virulence evidently prepared the reticulum cells for such a transformation. The replacement of the expended reticulum cells was retarded as a result of the second massive antigenic stimulus. The most likely hypothesis is thus that the manifestations of immunological depression are evidently associated with delay in the reproduction of reticulum cells capable of undergoing transformation into antibody-forming cells, i. e., plasma cells.

SUMMARY

Superinfection, induced in guinea pigs with a large dose of *R. burneti*, was accompanied by a brief rise of the complement fixing antibody titer (on the 6th-12th day) and a subsequent drop (on the 12th-50th day) and a new rise (on the 60th day). Morphological changes in the lymph nodes and spleen were characterized by a turbulent but brief proliferation of reticulum cells for a period of 2-4 days with subsequent rapid transformation of most of them into plasma cells. In this way it seemed as if the stocks of reticulum cells, capable of such transformation, were expended. The quick and almost simultaneous maturation of plasma cells leads to their rapid disappearance by degeneration. Temporary atrophy of reticular tissue especially noticeable in the medullary zone of the lymph nodes, resulted. The author considers that this temporary atrophy is connected with the state of immunological depression. The latter evidently results from the inhibition of the processes involved in the formation of reticulum cells, capable of transformation into antibody-producing cells, i. e., into plasma cells.

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*Original Russian pagination. See C. B. translation.