HISTOCHEMICAL CHARACTERISTICS OF MORPHOLOGICAL REACTIONS ENCOUNTERED IN BRUCELLOSIS

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Morphological studies of the pathogenesis and immunogenesis of Brucella infection have shown that resistance to infection is based on a phagocytary defense mechanism [4, 5].

Cytomorphological studies of the reaction to live vaccines have shown that the development of active immunity to brucellosis is associated with a strong macrophage reaction and with hyperplasia of elements of the reticulo-endothelial system; such a reaction should necessarily involve biochemical changes in the cells of the reticulo-endothelial system.

We have concentrated our attention on the study of nucleic acids, in view of the importance of the role played by them in the vital activities of cells.

Numerous histochemical investigations have established the importance of the part played by nucleic acids in the synthesis of proteins and the growth and division of cells.

The work of Ehrlich, Drabkin, and Forman [12] has revealed the relation between production of antibodies and changes in the nucleic acid content of lymph nodes located near the site of injection of vaccine. Maximum content of ribonucleic acids in the cells coincided with maximum production of antibodies in the lymph glands.

T. Harris and S. Harris [13, 14] obtained similar results. They also report the interesting observation that rise in cell ribonucleic acids does not take place following introduction of nonantigenic substances (lanolin, oil emulsions).

Papers on the distribution of nucleic acids in the presence of an active tuberculous process have appeared of recent years in Soviet journals.

A. Ya. Fridenshtein [10] and M. M. Averbakh [1] have shown that during the early stages of development of the inflammatory process in tuberculous infection hyperplastic reticular and other cells become enriched in ribonucleic acid, but with further development of the tuberculous lesions the ribonucleic acid content of the cytoplasm of the cells diminishes.

M. P. Pokrovskaya [8] has demonstrated the existence of a mutual relationship between phagocytary processes and cell ribonucleic acid content.

On the assumption that the content of nucleic acids in cells is correlated with functional state of organs and tissues, we investigated the amount and topographic distribution of ribo- and deoxyribo-nucleic acids in cells of the lymph glands and spleen, in infected and immunized animals.

We applied the Brachet reaction to the histochemical study of ribonucleic acids (RNA), and the Feulgen reaction to the study of deoxyribonucleic acids (DNA).

The present research includes data on three groups of guinea pigs: vaccinated, revaccinated, and infected. In all 100 animals were studied, at various times after vaccination or infection (1 – 2 – 5 – 10 and 30 days, and 3 and 6 months.)

The guinea pigs were vaccinated with the strain Br. abortus$_{19}$, at a dosage level of 10^9 cells, subcutaneously.

Subcutaneous revaccination was effected with the same strain, using a dose of 500 million cells, 3 and 8 months after primary vaccination. The animals were infected with the virulent strain Br. melitensis$_{548}$, introducing a dose of 10 cells.

The following results emerged from a study of the distribution of RNA and DNA in cells of the reticulo-endothelial system, during development of the vaccinial process.

During the first few days after vaccination with a dose of 1 billion microbial cells we observed a generalized lymphoid hyperplasia, with sporadic clear cells, in the lymphatic glands and spleen. This morphological reaction was not associated with change in the RNA and DNA contents of the cells. The reticular cells contained very little RNA. As in normal animals, RNA was found chiefly in the lymphoblasts and lymphocytes.

The RNA content of the cells changed with increasing intensity of the reaction in the lymph nodes and spleen, characterized by increase in the size of centers of multiplication of follicles of lymph glands, and by generalized large cell hyperplasia. Large numbers of reticular cells, with a high RNA content of the cytoplasm, and with well-defined nucleoli, appear at these centers.

The number of lymphoblasts containing RNA in the cytoplasm increases. On the 15 – 30th day after vaccination isolated small nodes of reticular and epithelioid cells make their appearance, against a background of large-cell hyperplasia. The cytoplasm of reticular cells becomes impoverished in RNA during their transition into epithelioid cells. Sporadic reticular cells, which lose their connection with the reticular stroma, change into polyblasts, and have RNA-rich cytoplasm. The phagocytary function of these cells (macrophages) is, however, associated with fall in cytoplasmic RNA content.

At longer times after vaccination (6 months) the reticular cells of the lymph nodes and spleen have little RNA in the cytoplasm, but a high content in the nucleoli.

The plasma cells are of particular interest. By the 10 – 15th day after vaccination large numbers of plasma cells are encountered in the lymph glands and spleen. In the earlier post-vaccinial stages juvenile forms of plasma cells are seen, with a scant cytoplasm of a high RNA content, but as the process of generalized cell hyperplasia develops the total volume of protoplasm also increases, and together with it its RNA content.

The plasmocellular reaction in the lymph nodes is now related to synthesis of antibodies. A. Fagreus [11], in his painstaking researches, has demonstrated the relationship between increasing basophilicity of plasmocytes and their antibody content. The presence of plasma cells with a strongly basophil protoplasm, after immunization with live antibrucellosis vaccine, merits special attention, since we have encountered this type of reaction only during development of the vaccinial process.

A rapid response reaction is seen in the reticulo-endothelium of lymph glands and the spleen after vaccination of guinea pigs, 3 and 8 months after primary vaccination.

A pronounced large cell hyperplasia, with numerous mitoses, and formation of macrophages, is evident in the lymph glands within 2 hours. Large numbers of polyblasts and plasma cells make their appearance. Similar effects are seen in the spleen.

This morphological reaction is associated with accumulation of RNA in these cells. Its content rises considerably in the cytoplasm of the reticular cells (Fig. 1). The nucleolus present in the nuclei of these cells increases in size, and contains very much RNA. Cells containing two or three distinct nucleoli are very frequent.

Such an enrichment of the reticular cells with RNA points to their exceptional activity. Reticular cells displaying phagocytary activity lose part of their RNA in the cytoplasm, but they still have a higher RNA content than do the macrophages encountered during primary immunization. Other cells present in the lumph nodes,

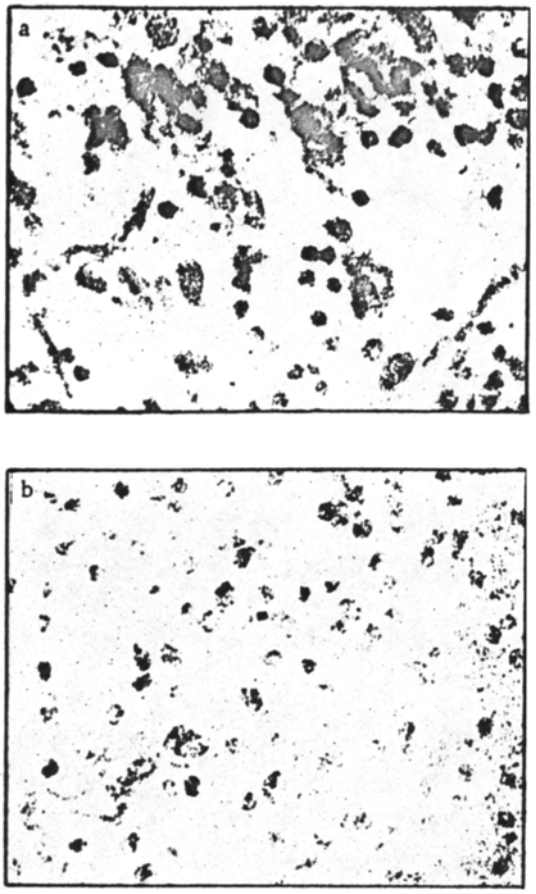

Fig. 1. Lymph gland of a revaccinated guinea pig. a) reticular cells, with a high RNA content; b) the same section, treated with ribonuclease. Magnification × 660.

viz., lymphoblasts, polyblasts, and plasma cells, are the richest in RNA (Fig. 2). The distribution of RNA within the lymphoblasts is very often irregular. During the stage of intensive hyperplasia of the follicles at proliferation centers we encountered numerous macrophages containing large spheroidal inclusions, which stained deeply with pyronin, in contrast to the faintly staining cytoplasm. These inclusions were absent after treating the sections with ribonuclease, showing that they consisted of RNA. It is not yet clear whether this RNA originated from the macrophage itself, or whether it represents phagocytized cell debris. We were unable to find any significant shifts in the DNA contents of the cells. It should, however, be noted that the DNA content of the lymphoid cells increased as the vaccination process developed. The DNA content of the nuclei of the reticular cells also increased somewhat, while the epithelioid cells contain practically no DNA, the little there is being distributed in the form of very small granules at the peripheries of the nuclei. The DNA content of the nuclei rises during the process of mitotic division of the cells.

Simultaneously with the vaccinated animals, we observed a group of guinea pigs infected with a virulent culture of the strain Br. melitensis$_{548}$ (inoculation dose 10 microbial cells). We studied the distribution of nucleic acids in elements of the reticulo-endothelial system during development of the infection.

During the first 5 – 10 days after inoculation we observed a generalized lymphoid and large cell hyperplasia of cells of the reticuloendothelium of lymph glands and of the spleen, with approximately the same RNA contents as were encountered in primary vaccination. The RNA content of the cells then fell, as the pathological process developed, with formation of large epithelioid proliferates, occupying 90-95% of the lymph nodes, and forming specific granulomata. The epilthelioid cells all had a low RNA content, and RNA was practically absent from epithelioid and giant cells of the specific granulomata, and diminished in the lymphoblasts.

The nuclei of most of the epithelioid cells either had no nucleolus, or had only a faintly discernible one.

The data on the distribution of RNA in elements of the reticuloendothelium of infected animals indicate that the functional activity of these cells is considerably lowered.

Our cytological study of formation of immunity to brucellosis showed that high and stable immunity can be achieved with a dose of 10^9 living microbial cells. Formation of immunity is associated with an intense macrophage reaction, and with vaccinial hyperplasia of elements of the reticulo-endothelial system, which is most pronounced on the 10 – 15th day after vaccination.

By that time the guinea pigs are immune to infection with virulent strains of Brucella.

The hyperplasia observed in the reticulo-endothelium during the development of the vaccinial process taking place after inoculation with 10^9 microbial cells is associated with accumulation of ribonucleic acid in the cytoplasm and nucleoli of reticular cells.

The RNA content of all the cells of lymph nodes and of the spleen is at a maximum on the 15 – 30th day after primary vaccination, and on the second day after revaccination.

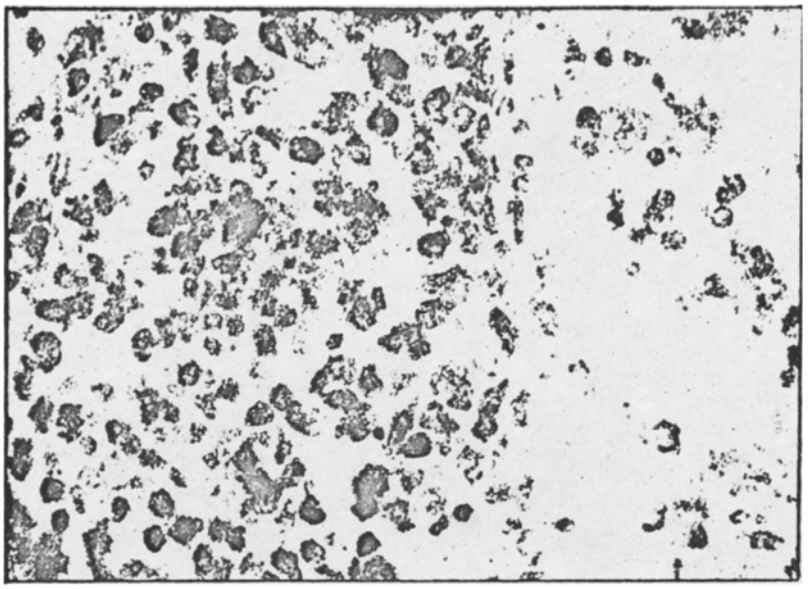

Fig. 2. Lymph gland of a revaccinated guinea pig. Plasma cells, rich in RNA. Magnification × 660.

Inoculation of guinea pigs with a virulent strain of Br. melitensis leads to an infection causing pronounced pathological changes in the organs, associated with a fall in the cell RNA content.

Thus the hyperplasia of reticulo-endothelial elements, observed in response to live vaccine is associated with increase in the RNA content of reticular cells, pointing to the enhanced functional activity of these cells in the synthesis of proteins.

The work of B. V. Kedrovsky [6, 7], A. N. Belozersky [2], G. I. Roskin [9], G. Casperson, J. Brachet [3], A. Fagreus [11], and others has provided convincing evidence of the direct connection of ribonucleic-acid with the synthesis of proteins and specific antibodies.

We are justified, on the basis of the work of these authors, in drawing the conclusion that vaccinial hyperplasia of reticulo-endothelial elements, in response to live vaccine, involves intensification of cell metabolism, leading to reconstruction of intracellular enzyme systems and to formation of specific globulins.

SUMMARY

Production of immunity in brucellosis as a result of introduction of live vaccine is associated with severe macrophagic reaction, as well as with vaccinial hyperplasia of reticulo-endothelial elements.

Intensive accumulation of ribonucleic acid in the cytoplasm and in the nuclei of reticular cells takes place during the process of vaccinial hyperplasia of reticulo-endothelium. Phagocytic function of macrophages is connected with reduction of ribonucleic acid in their cytoplasm. The largest quantity of ribonucleic acid in all the cells of the lymph nodes and the spleen is noted within 15 to 30 days following initial vaccination. The same picture is revealed on the second day after revaccination which is carried out in 3 months.

Brucellosis induced in guinea pigs by virulent culture of Br. melitensis causes pronounced pathological changes in the organs and is associated with reduction of content of ribonucleic acid in the cells.

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* In Russian.
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