MORPHOLOGY AND PATHOLOGICAL MORPHOLOGY

CONCERNING THE DISTRIBUTION OF NUCLEIC ACIDS IN SPECIFIC GRANULOMAS DURING EXPERIMENTAL TUBERCULAR INFECTION AND VACCINATION PROCESS

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Numerous works, performed by histochemical methods, enable us to establish morphological criteria for determining the varying states of the synthesis of albumins in the cells of most animal tissues. It has been shown that the distribution of nucleic acids is one of the most significant criteria of the functional state of nuclear elements and especially of those which participate in the plastic tissue processes [1, 2, 6].

The overwhelming mass of the gathered histological data is concerned with the embryological and regenerating activities of the tissues, and there are only scattered investigations dealing with the pathological changes of the tissues, although the need for such studies is obvious.

In the present communication there are presented observations on the distribution of the nucleic acids in specific tissue granulomas, developing in the course of tuberculous inflammation. Concepts concerning tuberculous granulomas as a protective structure of the organism were first advanced by I. I. Mechnikov, and suggested a study of the functional state of the cell elements and their fundamentals, i. e., epithelial cells. Already investigations by ordinary morphological methods show that under varying conditions and at various stages of the specific inflammation the functionaly state and the direction of development of the cellular elements of the tuberculous granuloma are dissimilar. For a true evaluation of these structures it is essential to obtain comparable histochemical data at varying stages of the specific inflammation, as when the protective reaction of the organism proves adequate or inadequate for the overcoming of the infection.

By itself the content of nucleic acids cannot serve as an indicator of their role in the protective tissue reaction and the genesis of immunity. The changes in the content of nucleic acids which appear during the course of the infection are considered pertinent. With this, while evaluating the cellular nucleic acids, we must conduct comparisons within the known limits of cellular structure of those elements which carry the genetic and functional similarities. In this case it is the reticular cell, polyblast, epithelial cell, macrophage, fibroblast. To compare these elements with arbitrarily selected cells, rich in ribonucleic acid (e. g., with plasmacytes), as is sometimes done, would be incorrect. Evaluations based on such comparisons cannot characterize the true functional condition of the cellular elements of specific granulomatous tissue.

We will present material concerning the distribution of nucleic acid in the cellular elements of specific granulations in guinea pigs, vaccinated with BCG* infected with a virulent TB culture Rv-44 and attenuated tuberculous strain.**

*Vaccine strain Calmette-Guerin.

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The investigations were conducted parallel with the usual histologic studies of the same objects – lymphatic nodes of guinea pigs. The material was fixed in alcohol for staining by the method of Brash and in Zenker-formalin for staining by the method of Feulgen. The animals were sacrificed at various intervals after infection or vaccination (1 week to 6 months), which enabled us to follow the dynamics of the specific inflammatory process. Altogether the lymphatic glands of 45 guinea pigs were studied.

Parenteral vaccination of guinea pigs with BCG (supradermal and intradermal in doses of 20 and 0.01 mg, respectively) produce a vaccinating process and formation from the 3rd week of specific granulomas in the regional lymph nodes [3, 5]. This specific granulation tissue arises principally by the conversion of reticular elements of the lymph node stroma into epithelioid cells, although a part of these latter, possibly, are formed also from lymphoid elements. The histogenesis of the clumping goes through several stages and is connected with changes in the functional condition of the cellular elements, which are reflected in the nucleic acid distribution in them. The vaccine rods reaching the lymphatic nodes are phagocytosed by the reticular cells, which are hypertrophied in the process: their enlarged cytoplasms is enriched with ribonucleic acid. In the nuclei of the reticular cells, in which the desoxyribonucleic acid is usually contained in the form of small granules under the nuclear envelope, there appears a pronounced large nucleolus of complex structure, consisting of ribonucleic acid and large granules of desoxyribonucleic acid. This nucleolar formation points, as is known [6], to intensified synthesis of albumin in the cell. These cellular elements with a phagocytic function are the first stage in the formation of the typical epithelioid cells from the reticular; they correspond to the specific polyblasts (see the Figure).

The mature epithelial cells have a rounded form and a sharply contoured homogeneous cytoplasm. The nucleus of the epithelioid cell retains the clearly distinctive large nucleolus; the cytoplasm contains ribonucleic acid, but markedly less then do the reticular cells – the polyblasts. An especially large quantity of ribonucleic acid is contained in the nucleoli of the epithelioid cells in the region of intensive formation of reticular whorls in the granulating tissue. In such cells there is also evidenced an increased amount of ribonucleic acid in the cytoplasm.

In mature granulomatous tissue the epithelioid cells are not alike in the sense of nucleic acid distribution: among them are encountered elements extremely rich in ribonucleic acid, as a consequence of which they stain conspicuously with methyl green pyronin. The significance of these cells has not as yet been clarified.

The epithelial cells of the granulomas originating from BCG introduction have several developmental pathways (see Figure): they may become transformed into fibroblastic elements with redistributed nucleic acids, diminution of the amount of ribonucleic acid in the cytoplasm and nucleolus, and also by the disappearance of the large nucleolus and the scattering of the desoxyribonucleic acid in the form of minute particles under the nuclear membrane; a part of the epithelioid cells can change into motile, actively phagocytic macrophages, engulfing cellular fragments. With this their nuclei lose the complex nucleolus, the desoxyribonucleic acid scattering under the nuclear membrane, while in the cytoplasm there accumulates a large amount of ribonucleic acid. The dissolution of the whorls of the vaccinating process is connected with the change of a part of the epithelioid cells into reticular, this being connected with accompanying mitoses, the disappearance of the complex nucleolus and a marked diminution of the amount of ribonucleic acid in the cytoplasm.

On the basis of nucleic acid distribution the multinucleated cells of the granulomatous tissue can be divided into two groups: 1) cell elements of the Langhans type, which have big nuclei with large nucleoli, containing a considerable quantity of ribonucleic acid, the cytoplasm possessing a relatively scanty amount of ribonucleic acid; 2) elements of the multinucleated symplast type with insignificant nuclei without definite nucleoli, with a very large amount of ribonucleic acid in the cytoplasm (chiefly on the periphery of the symplast). These differences point to varying functional states of the giant cells.

On the whole the distribution of the nucleic acids in the cellular elements of the granulomatous tissues testifies to their functional activities and capacity for survival during the vaccination process. A state of intensified albumin synthesis, which characterizes the epithelioid cells, speaks either for cellular growth and active participation in plastic processes, characterizing granuloma formation, or for a storage in them of albuminous material which, possibly, plays a definite role in immunogenesis.

Infecting guinea pigs with 0.00001 mg of a virulent TB strain leads to the appearance in the lymph nodes of granulomatous tissue which goes through the same stages of development as the granulomatous tissue of the

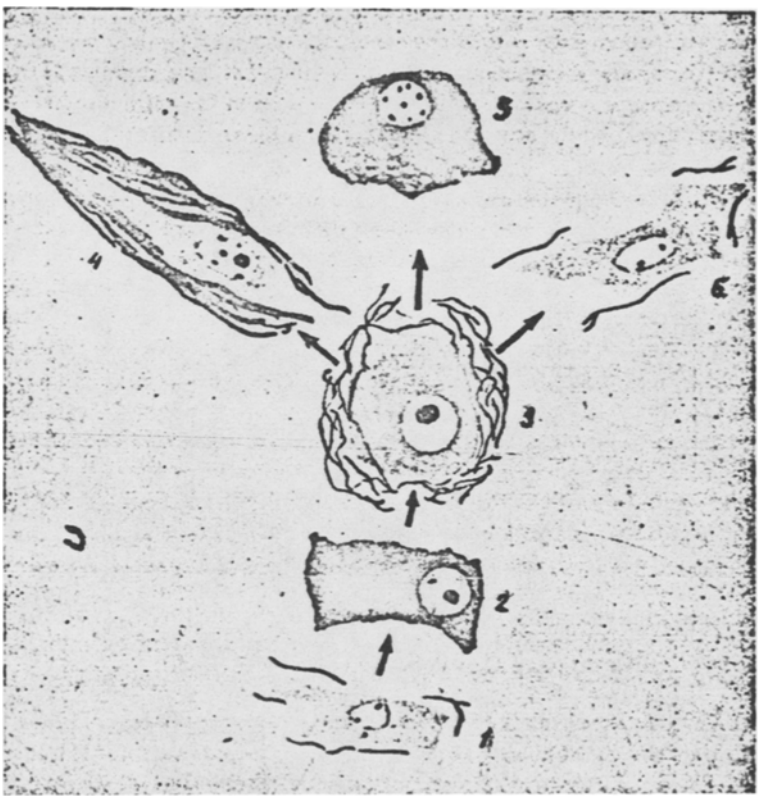
vaccinating process. In these, however, necrobiotic changes soon occur, up to caseation. At specific points, at the same time, there can take place sclerotic processes, but these do not lead to complete isolation of the destructive foci and the process continues to spread, sclerosis occurring as a result of the growth of nonspecific connective tissue rather than from a transformation of specific elements of the granuloma itself.

The epithelioid cells of a granuloma when infected with a virulent TB strain, in most instances quickly lose their nucleoli; their nucleus swells with the small granules of desoxyribonucleic acid under the cell membrane; the quantity of ribonucleic acid in the cytoplasm sharply diminishes. Similar events take place in the Langhans cells. In the granulomatous tissue a very small number of epithelioid cells have a cytoplasm rich in ribonucleic acid.

Virulent tuberculous rods manifest, apparently, a depressing, toxic influence on growing granulomatous tissues, and in guinea pigs its elements lose their capacity for survival. Such cells do not participate in the plastic processes and in the isolation of necrotic centers. Cytochemically their functional inadequacy is displayed by the disappearance of the nucleolus and the ribonucleic acid in the cytoplasm.

As already described [4], infection of guinea pigs with TB bacteria of diminished virulence causes the development of a chronic, self-healing tuberculous process, not causing death of the animals. There occur characteristic changes in the morphology of the specific inflammation; in a tissue reaction appear changes of sclerosis and demarcation, with the active participation of the epithelioid cells in the plastic processes, forming whorls and changing into elements of the fibroblastic type. In such granulomatous tissue a large fraction of the epithelioid cells is rich in ribonucleic acid and possesses large nucleoli.

During fibroblastic transformation the epithelioid cells are clearly differentiated from the fibroblasts of connective tissue by the long maintenance of the large, complex nucleolus and the high ribonucleic content of the cytoplasm. This confirms the qualitatively different sclerotic changes that ensue with or without epithelioid cell participation.



Scheme of the transformation of cellular elements during specific inflammation, produced by BCG; 1) Reticular cell; 2) polyblast; 3) epithelioid cell; 4) fibroblastic element; 5) macrophage; 6) reticular cell.

The majority of the elements of granulomatous tissue keep a large amount of ribonucleic acid in their cytoplasm even in the event of distinct necrotic foci separating out. With this the epithelioid cells near the necrobiotic centers survive longer than the nonspecific fibroblasts. All this can be regarded as a manifestation of the specific stability of the epithelioid cells in given conditions as described.

During infections both from virulent TB rods and from attenuated strains, even in close proximity to necrotic foci there are seen many plasmacytes containing large amounts of ribonucleic acid in the cytoplasm, and there is no ground for a belief that they play any significant role in the protective tissue reaction caused by tuberculosis.

The considerations advanced permit only preliminary conclusions, as the nucleic acid content was estimated without quantitative data. It seems evident that the transformation of reticular cells into epithelioid is bound up with a ribonucleic acid content enrichment; this points, in all probability, to a heightened activity and a functional specialization of these elements. Histochemical reactions testify to a diversity among the epithelioid cells of the granulomatous tissue. Among them are met elements with a sharply elevated ribonucleic content of the cytoplasm, at the same time there being noted that the number of these cells varies under varying conditions of the specific process; in part, when the infection is with a less virulent strain, there being more of them than with the introduction of more virulent strains.

The main mass of the epithelioid cells in young granulomatous tissues during the course of a tuberculous infection as well as a vaccination response has a smaller quantity of ribonucleic acid in the cytoplasm than do the nonspecific polyblasts, but contains it in the large, complex nucleolus, i. e., it possesses the apparatus for intensified albumin synthesis. These are cells with plastic functions, this functional activity manifesting itself especially in intensified cellular membrane activity, which confirms the active participation of epithelioid cells in fibrillogenesis.

The wide range of transformations to which epithelioid cells can adapt themselves is measurable by changes in their functional activity and their content of nucleic acids. Especially significant is the capacity of the epithelioid cells during the vaccinating process to change into reticular cells (which may be connected with immunogenic mechanisms) and into fibroblastic elements in those cases when the tissue response is definitely of a defensive character. In sclerotic processes, associated with transformations of the epithelioid cells themselves, there ensues a more valuable demarcation, and the scarring elements show more viability than with sclerosis by way of nonspecific scar-forming tissues.

All this data testifies to the protective nature of the tissue reaction during a tuberculous infection and of the important role in it of plastic processes. They show that the nucleic acid content is a definite gage of the functional state of the cellular elements during specific inflammations.

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