EXPERIMENTAL BIOLOGY

TISSUE CELL CHANGES IN HUMAN LYMPHATIC GLAND EXPLANTATES

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Accounts of changes in human lymphatic gland tissues during explantation are very scanty; they are limited in fact to a series of papers, already quite old, which appear in various existing reviews [7, 9, 17] and monographs [12]. The authors have cultivated particles of lymphatic glands in order to solve certain specific problems (movement, rate of migration of lymphocytes and so on), without dealing at all with the growth characteristics of the cultures, the relationships between their component cells, and the ability of these cells to undergo change and differentiation. As test objects they used only normal lymphatic glands of rabbits [7, 8], apart from only one investigation devoted to culture of material from lymphatic glands of human fetuses [11].

It is not yet established with certainty whether the lymphocyte of the blood and lymph of different animals and man is a cell of low differentiation capable of progressive development, or whether it must be regarded as a cell with a specialized and diminishing power of conversion into other cell forms [1, 2, 4, 5, 10, 13-16, 19, 20]. This question has frequently been discussed but remains far from solved. When working with explantation of a leucocyte film the possibility of confusion by conversion of lymphocytes and monocytes cannot be excluded [24]. Explantates of lymphatic glands have the advantage over cultures from leucocyte films that monocytes, numerous in the latter, are practically absent.

The study of the hemopoietic organs, especially the lymphatic glands of the adult human subject, appeared therefore to be of great interest.

EXPERIMENTAL METHOD

The material cultivated consisted of fragments of the cortex of lymphatic glands, obtained from patients aged from 27 to 74 years, admitted to the First and Second Surgical Clinics of the Institute of Oncology. Cultivation took place in flasks. The solid phase was composed of heparinized chick plasma, Tyrode fluid and chick embryonic extract; the fluid phase — human serum to which was added Tyrode fluid. In all we carried out 24 series of experiments with 12 to 16 cultures in each. The cultures were studied for 45 days, whether living or whether at a standstill. Fixation was by 10% formalin, Carnoy's fluid and Zenker's formol. Staining was by Carazzi's or Heidenhain's hematoxylin, and by azure-eosin. To demonstrate argyrophilic fibers, the sections were fixed and impregnated with silver nitrate by Foot's method. Lipids were stained by Sudan III and Nile blue sulfate. Parallel studies were carried out on the original material used for the cultures. The explantated fragments usually consisted of cells of the reticular stroma with argyrophilic fibers, a small number of fibroblasts in the thickness of the trabeculae and capsule, and also along the course of the vessels, a large number of free lymphocytes (small and of medium size), and a few lymphoblasts distributed among the loops of the reticular stroma. Sometimes plasma and lipoid cells were encountered.

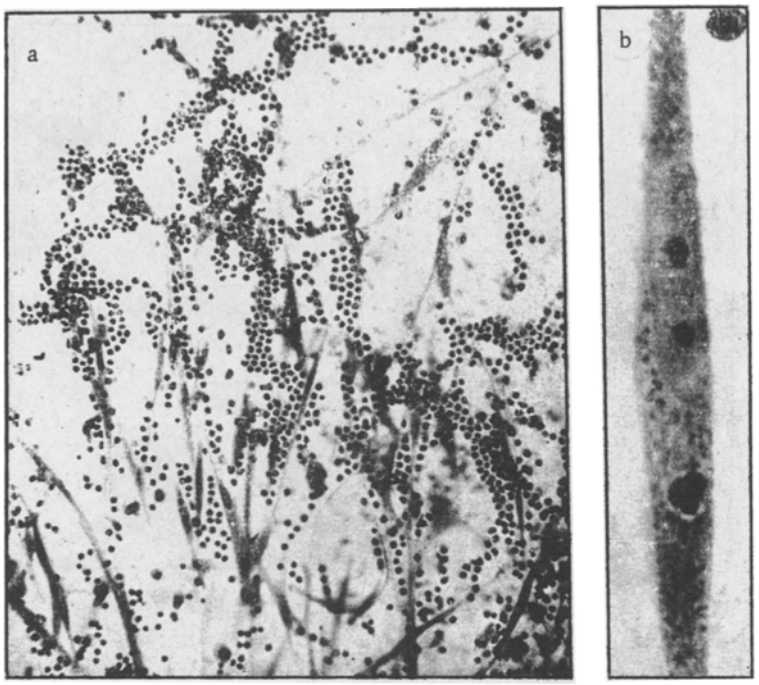

Fig. 1. Reticular cells and collections of lymphocytes in the zone of growth of an 8-day culture of the lymphatic gland of a 66-year old woman.
a) Total preparation. Formalin. Carazzi hematoxylin. Magnification 200 x. Basophilic granules and a phagocytosed lymphocyte in the cytoplasm of a reticular cell; 8-day culture of the lymphatic gland of a 66-year old woman; b) total preparation. Formalin. Caracci hematoxylin. Magnification 1300x.

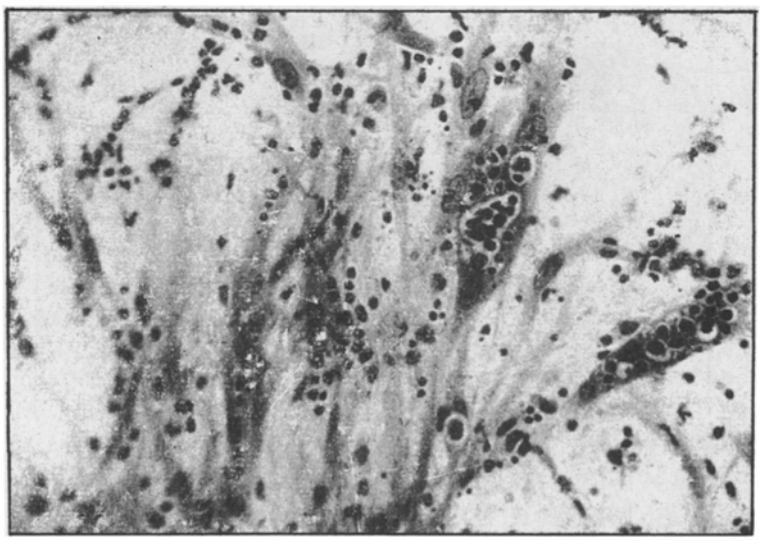

Fig. 2. Growing reticular syncitium with phagocytosed lymphocytes; 8-day culture of the lymphatic gland of a 42-year old woman; total preparation. Formalin. Carazzi hematoxylin. Magnification 1300x.

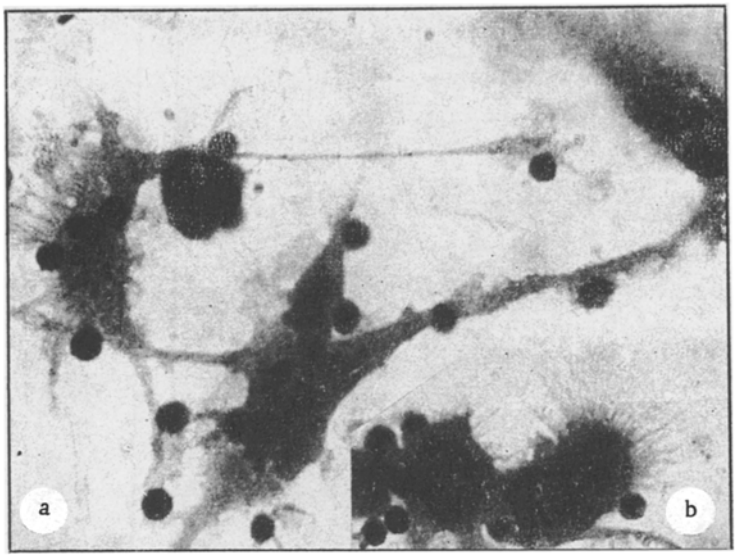

Fig. 3. Formation of "vesicles" (a) and axopodia (b) by reticular cells with phagocytosed lymphocytes; 4-day culture of the lymphatic gland of a 48-year old woman. Total preparation. Formalin. Carazzi hematoxylin. Magnification 1250x.

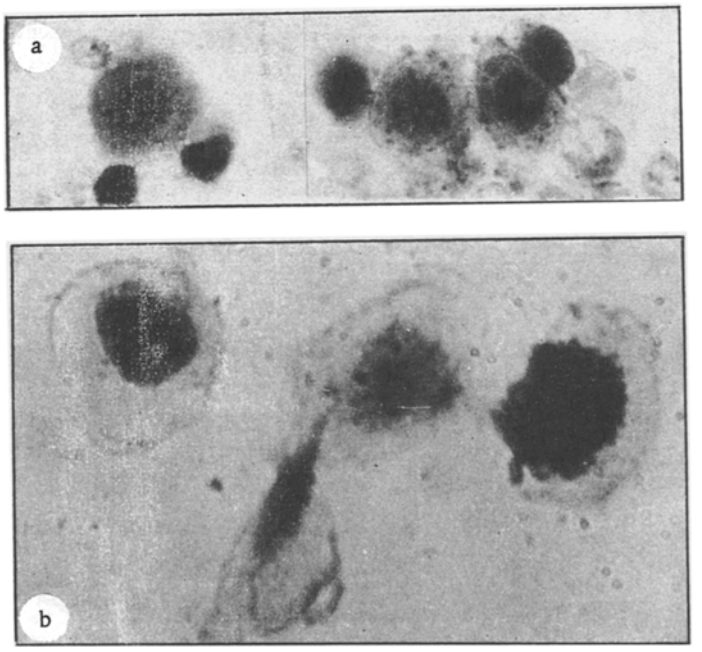

Fig. 4. Mitoses in polyblasts; 9-day culture from the lymphatic gland of a 35-year old woman (a); total preparation. Formalin. Carazzi hematoxylin. Magnification 1300x. Epithelioid cells with lipoid inclusions; 9-day culture from the lymphatic gland of a 35-year old woman (b). Total preparation. Formalin. Carazzi hematoxylin. Sudan III. Magnification 600x.

EXPERIMENTAL RESULTS

Twenty-four hours after explanation, the tissue fragment is surrounded by a wide zone of migration of lymphocytes. At later periods of cultivation the number of these cells in the zone of migration shows a marked increase; at the same time death of a high proportion of them takes place. At the end of the second and beginning of the third day, cells of the reticular stroma — spindle-shaped, stellate or having numerous processes — appear in the fibrin coagulum. Proliferation of these cells is sometimes accompanied by intensive fibrinolysis. They multiply intensively by mitosis, and after 5-6 days produce a quite extensive zone of growth, very reminiscent of the zone of growth in cultures of loose connective tissue [6, 27].

Alongside the growth of fibroblast-like reticular cells, proliferation of the relatively few fibrocytes of the trabelulae also takes place.

The structure and character of the zone of growth of connective and reticular tissues have much in common, which from time to time makes the picture extremely difficult to interpret. In such cases the criteria are the well marked syncitial structure of the reticular tissue and certain morphological and physiological properties of its component cells. It is also very difficult to distinguish the endothelium of the sinuses from the reticular stroma and the endothelium of the vessels from the fibroblasts of the trabeculae and capsule. This difficulty, which was previously mentioned by Grossmann [22], is particularly evident in cases of mixed growth. The difficulty in distinguishing between these cells depends on the properties, mentioned above, of the stroma of the lymphatic gland, and also on the fact that the endothelium of the vessels contains highly friable structures in addition to its complex membranes [18].

One very often observed characteristic feature of lymphoid tissue in tissue culture is the intimate relationship between the cells of the reticular stroma and the lymphocytes. The latter, situated in the loops of the reticular syncitium and in close contact with its cells, remain viable for a long time. Thanks to some influence (possibly of the nature of chemotaxis or thigmotropism) many of these remain for the whole time in direct contact with the reticular cells. Some of them lie so closely up against them that they appear to be adherent to the surface of the body or processes of these cells (Figs. 1, a and 2). When they migrate in the fibrin, the lymphocytes often form characteristic groups, shaped like narrow bands, round collections or garlands with branches in the form of trees (see Fig. 1, a). Structures to some extent similar were described by Hueper and Russell [23] in cultures of white blood cells as capillary-like formations. However, a careful study of these collections of lymphocytes makes it clear that they have nothing in common with endothelial vascular tubes but show their own distinctive nature and pattern of aggregation with each other, and their association with the cells of the reticular syncitium.

Reticular cells, which together with lymphocytes account for the bulk of the zone of growth in cultures of lymphatic glands, have a varied external appearance and a varied structure, which depend not only on the properties of the tissue itself but also on the conditions of its existence. Reticular cells growing in a fibrin coagulum are spidle shaped or have numerous processes. They are most commonly slightly elongated and have an outward resemblance to fibrocytes, which gave certain authors [7, 8, 11] grounds for identifying them with the fibrocytes of connective tissue. Reticular cells have a well-outlined cell body, a pale nucleus, poor in chromatin and usually with 1 or 2 nucleoli, and feebly basophilic protoplasm. Often it shows numerous vacuoles and basophilic granules (Fig. 1, b) and also a small number of droplets of fat.

Under certain conditions (a large quantity of decomposition products in the zone of growth, liquefaction of fibrin) some of the reticular cells lose their connection with each other and are converted into free, ameboid macrophages. When they migrate along the film into the cavity of liquefaction, the reticular cells show great variation in size and shape. The majority of them have feebly basophilic cytoplasm. This has a well-marked foamy structure, with numerous processes and vesicular formations with translucent, watery contents (Fig. 3, a).

In other cases, in a state of retraction, the cytoplasm of the cells of the reticular syncitium sometimes forms numerous thin, pale processes which resemble axopodia (Fig. 3, b). Both the attached and free reticular cells possess well-marked phagocytic properties and they vigorously engulf dead and living lymphocytes. The lymphocytes are situated in the loops of the reticular syncitium and as a rule are surrounded by a light rim — a digestive vacuole (see Fig. 2). In the course of time the phagocytic properties of the reticular tissue in the cultures grows weaker, although they are sometimes preserved until late periods of cultivation. Mitoses are often seen in the actively phagocytosing cells. Multinuclear giant cells, developing from reticular cells, are rarely

encountered; in the whole of our material we were able to find them in 3 series of experiments, but not in every culture and in small numbers. Multinuclear cells developed mainly in the liquefaction cavities on the surface of the film, but they were also to be found in the fibrin coagulum. In isolated cases the multinuclear cells attained a considerable size and contained 10 or more nuclei.

Within a day or two of explantation the lymphocytes begin to undergo changes. Being the least stable cells in these cultures, they very rapidly undergo necrobiotic changes in large numbers. The fate of the lymphocytes which remain viable is as a rule varied: some of them remain visibly unchanged until the last days of observation, while others — mainly those situated between the reticular cells and on their surface — undergo further progressive changes. These changes in the lymphocytes begin on the 5th-6th day of cultivation. Some of them become round and larger in size. The basophilia of their cytoplasm becomes more intense and their nucleus becomes excentrically situated and acquires the characteristic structure of plasma cells. The plasma cells seen in the cultures on the 6th-8th day after explantation are undoubtedly formed there, for they are only rarely found in the original material and furthermore they die in the course of the first 2-3 days.

Development of plasma cells is observed in few cultures (1-2%). The bulk of the lymphocytes develop into macrophages. In both the unchanged lymphocytes and in the macrophages formed from lymphocytes many mitoses are seen. Some of these cells become binuclear as a result of amitotic division of the nucleus. Sometimes fragmentation of the nucleus is seen. The increase in the number of macrophages in the zone of growth takes place on account of the continuing conversion of lymphocytes and of mitotic division. Mitoses are especially numerous in the 8-9-day cultures (Fig. 4, a).

On staining with Sudan III a large number of small droplets of fat are shown in the cytoplasm of the completely viable and intensively proliferating macrophages. The subsequent fate of the macrophages which form differs: some of them die after a brief existence, others, depending on conditions, are converted into other cell forms. A very small number of macrophages remains unchanged for a considerable length of time.

Conversion of macrophages into fixed cells of the fibroblast type is seen rarely (6-8%). In cultures where this occurs, a thin zone of growth forms, almost identical with that forming in connective tissue explantates. However the fibroblast-like cells, developing from lymphocytes, under certain conditions (cooling, mechanical injury and so on) become round again and become converted into free, round macrophages. The formation of multinuclear giant cells (by fusion) from macrophages is observed still more rarely. Often these giant cells do not differ from those formed from the cells of the reticular stroma and they appear in the central fragment and in the zone of growth, not only around foreign bodies but also where these are absent. Very often the macrophages are converted into cells which were described by a number of authors under the name of "epithelioid cells" [21, 25, 26]; they develop in those cases where the macrophages are spread out over the film in the cavity of liquefaction. Differing widely in their shape and size, the "epithelioid cells" retain to only an insignificant degree their powers of phagocytosis. The protoplasm of their bodies is always sharply divided into a central, endoplasmic part, strongly basophilic and containing the nucleus and a wide, transparent, almost colorless but clearly defined ectoplasm (Fig. 4, b). In the endoplasmic part are sometimes found solitary basophilic granules and a varying number of droplets of fat. Sometimes the "epithelioid cells" form long, slender and straight processes which connect them to each other.

Comparison of our own findings with those in the literature shows that the lymphocytes of the blood and lymphatic glands undergo similar changes in culture. Both develop into macrophages, fibroblast-like cells and "epithelioid cells." However in cultures of lymphatic glands, as also during aseptic inflammation [3], the conversion of lymphocytes into macrophages, "epithelioid cells" and fibroblast-like cells takes place more slowly and in much smaller numbers. In contrast to explantates from leucocyte films [4], "epithelioid cells" in cultures from lymphatic glands were never combined with each other inside epithelial-like membranes and they could never be seen to form multinuclear giant cells. In the conditions of our experiments the lymphocytes of the zone of growth were not converted into cells identical with connective tissue fibroblasts and after cultivation for 30-40 days no fibrous intercellular material was formed. The latter fact is in complete agreement with the findings of A. I. Zhudina [4], who studied the changes in the human white blood cells in tissue cultures. However, by itself it is far from opposing the fundamental views of A. A. Maksimov and of other workers, who consider that the lymphocytes are cells of low differentiation, capable of progressive development in various directions. The results which we have obtained is new evidence of the great powers of development of the lymphocytes of adult human lymphatic glands.

In cultural conditions a large part of the reticular cells retain their sessile character and resemble fibroblasts. In this condition, however, they are actively phagocytic and, at various stages of cultivation, they are converted into macrophages which is not characteristic of the fibrocytes of connective tissue, according to the view prevailing at the present time.

SUMMARY

The change of lymphocytes into macrophages, fibroblastlike cells and "epitheliod" elements was observed in culturing the tissue of human lymph node. Lymphocytes were not transformed into elements identical to fibrocytes of connective tissue and did not form the fibrous intercellular substance in 30-40 days of culturing. The majority of the reticular cells preserved its stationary nonameboid character and resembled fibroblasts. They exhibited an actively phagrocytic function and were transformed into macrophages at various stages of culturing.

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