EFFECT OF THE EPITHELIAL STROMA OF THE THYMUS ON DIFFERENTIATION OF LYMPHOCYTES

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It is known that the transplantation of the thymus in a diffusion chamber to animals thymectomized after birth restores the normal morphology of lymphoid tissue and the immunological competency of the organism. During cultivation of the thymus in a diffusion chamber only the epithelial stroma is retained. Apparently the epithelial cells of the thymus excrete humoral substances which stimulate the development of lymphoid organs of the thymectomized animal.

The purpose of this work was to study the contact effect of the epithelial stroma of the thymus on differentiation of lymphoid and hematopoietic cells in an isolated system.

EXPERIMENTAL METHODS

Lymphocytes from the thymus and lymph node and bone-marrow cells were secondarily transplanted in a diffusion chamber in which the epithelial stroma of the thymus was cultivated. We assumed that if the epithelium of the thymus had an effect on lymphocytes sustaining their proliferation and differentiation then transplantation of the lymphocytes to the prepared stroma in the diffusion chamber should lead to a longer preservation of the lymphoid structures than when the lymphocytes are in an empty chamber where they, as is known [2, 6], quickly lose their differentiation.

To prepare the diffusion chambers we used millipore filters type HA and TH 150 and 25 μ thick with a pore size of 0.45 ± 0.01 μ (see [1]). The filters were cemented to a plastic ring with an inside diameter of 1 cm and a wall height of 2 mm. In the wall of the ring was a hole, which, after primary and secondary transplantation of the tissue, was closed by a piece of the filter. The diffusion chambers were sterilized with 70° alcohol for 20 min.

The following groups of experiments were carried out (see table).

The work was performed on C57B1 mice. The diffusion chambers were transplanted intraperitoneally. Before transplantation to the second recipient the chamber was cleaned free from the connective-tissue capsule. Before fixation the chambers were freed from the surrounding tissue, the filters were removed from the ring and fixed with alcoholformol. From most of the filters we prepared whole mounts stained with hematoxylin or by means of the PAS method with hematoxylin counterstain. From other chambers we prepared paraffin sections which were stained with methyl green pyronin or hematoxylin-eosin.

RESULTS

On the 10-11th day of growth of thymus of newborn mice in the diffusion chamber the entire filter was covered with a monolayer of cells lying close to one another. These cells were of various shapes with distinctly

Scheme of Setting-Up the Experiments

No. of series	No. of chambers	Material for primary transplantation in chamber	Material for secondary transplantation in chamber	Period of secondary transplantation (in days)	Period of fixation (in days)
I	14	Thymus of newborn mice	–	–	10, 11, 22, 25
II	10	Spleen of newborn mice	–	–	10, 25
III	8	Thymus of newborn mice	Suspension of thymocytes of mature mice ($1.2 \cdot 10^6$ cells)	7	21
IV	6	One lobe of thymus of mature mice	½ lobe of thymus of mature mice	14	28
V	9	The same	Lymph node of mature mice	14	28
VI	3	"	Suspension of bone marrow of mature mice	14	28

outlined bubblelike nuclei. The cytoplasm was frequently foamy and had distinct outlines. The cells formed a unique membrane covering the entire filter. Against the background of this membrane were found small formations with cells concentrically arranged in several layers (Fig. 1) which, probably, were analogous to Hassall's corpuscle in the undamaged thymus. It is interesting that these formations were situated at comparatively regular distances from one another and thus uniformly covered the entire membrane. The foci had a different degree of maturity, i.e., they consisted of a larger or smaller number of cells; the diameter of the foci was 200 μ. In the younger foci we found many mitoses. Among the cells of the monolayer were giant cells with 1-2 large light nuclei. No lymphoid cells were detected on the filters.

On the 22-25th day of cultivation of the thymus of newborn mice in the diffusion chamber, cell accumulations of the Hassall's corpuscle type, in the center of which a zone of degeneration of the epithelium was distinctly noticeable, were retained. The cells of the membrane covering the filter changed – they became round, a vacuole appeared in the center, and the nucleus was displaced toward the periphery. In the center of the thymic corpuscles, in the cytoplasm of the cells composing the membrane, and between these cells we noted drops and granules of a PAS-positive substance.

On the tenth day of cultivation of the spleen of newborn mice, cells of a connective-tissue type lying in one or several layers were on the filters. These cells were very different morphologically: elongated, spindle-shaped, with thin long processes, numerous radiant processes, and distinct contours without processes. Among them we found groups of cells of the myeloid series. Their arrangement was extremely characteristic: 3-5 myeloid cells lay on

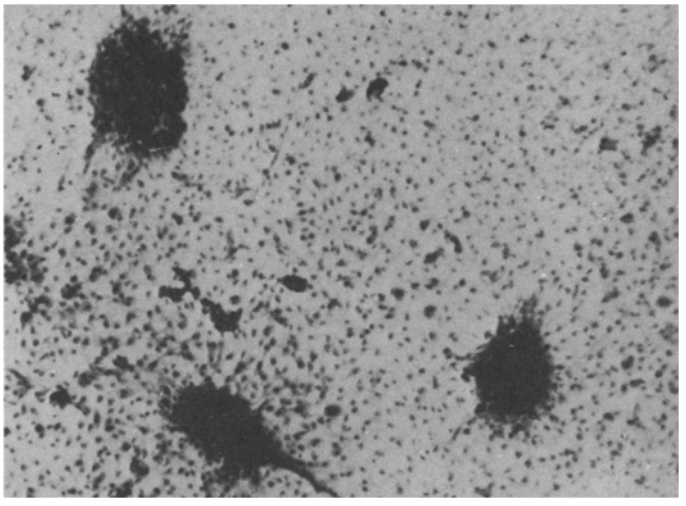

Fig. 1. Foci of Hassall's corpuscle type on filter. Series I. Period 10 days. Whole mount. Objective 8×.

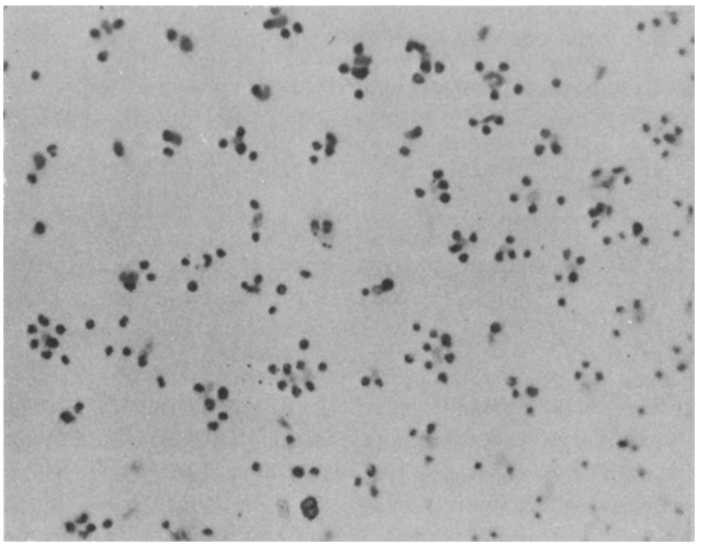

Fig. 2. Foci of myeloid cells around reticular elements. Series II. Period 10 days. Whole mount. Objective 8 ×.

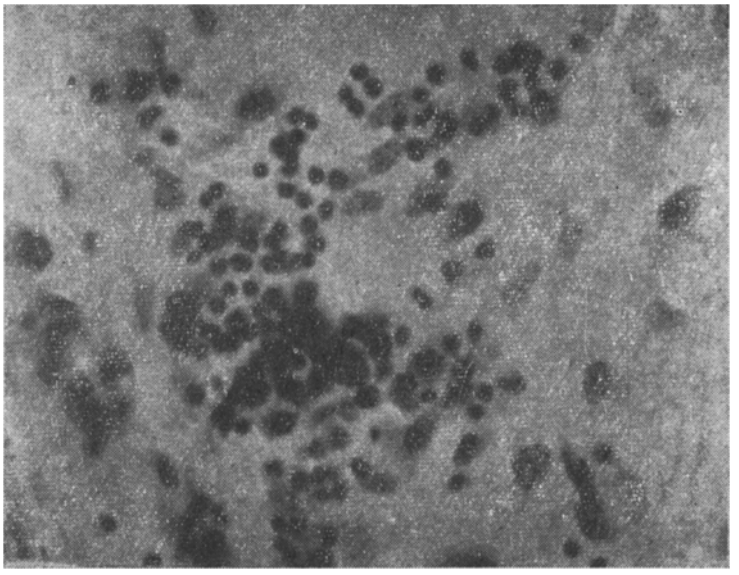

Fig. 3. Accumulations of lymphocytes on the thymus epithelium. Series V. Period 28 days. Whole mount. Objective 24 ×.

each stellate reticular cell. Myeloid elements were virtually absent in the spaces between the reticular cells. Since the reticular cells formed a uniform network on the filter, the entire structure acquired a very regular pattern (Fig. 2).

On the 25th day of cultivation of the spleen the filter was covered by infrequently located vacuolated cells of the macrophage type. Hematopoietic elements were not found among them.

Twenty-one days after transplantation of the thymus of newborn mice and 14 days after reinjection of thymocytes the filter was covered by 1-2 layers of cells, among which formations of the thymic corpuscle type were found. Epithelial cells of various morphology predominated among the cells of the monolayer. Here and there we found on the filter lymphocytes arranged separately or as accumulations. Connective-tissue elements and lymphocytes were found in the structures of the type of Hassall's corpuscles along with epithelial cells.

Upon cultivation of the thymus of a mature animal in the diffusion chamber and reinoculation of pieces of the thymus or lymph node of the mature donor, accumulations of lymphocytes (Fig. 3) and individual lymphocytes

among the cells of the epithelial stroma were found on whole mounts and sections. As a rule, the epithelium in the chambers was represented by a one cell layer without formations of the thymic corpuscle type.

If a suspension of cells of the bone marrow was applied to the epithelial stroma, there were no accumulations of lymphocytes.

Thus, upon transplantation of the thymus in a diffusion chamber a proliferation of the stroma occurs, the bulk of the elements of which have an epithelial nature. The formed system of Hassall's corpusclelike cell accumulations lying at an appreciable distance from one another occur against this background. These formations arise in the monolayer upon transplantation of the thymus of newborn, but not of mature mice. Lymphoid elements were not detected in the chamber after ten days. Upon reinjection of the thymus, suspension of thymocytes, or lymphocytes in the chamber with the prepared epithelial stroma the lymphocytes are retained and compact lymphoid accumulations form.

In the case of the cultivation of the spleen in an empty diffusion chamber no lymphocytes are detected by the tenth day. The reticular cells of the spleen by contact interaction maintain differentiation of the myeloid elements in the chamber the first ten days. In this respect the reticular stroma of the spleen differs from the stroma of the bone marrow. In the latter case, according to our data [3], the bone tissue and not the reticular tissue maintains differentiation of the myeloid cells. On the other hand, the reticular cells of the spleen do not have an apparent effect on differentiation of lymphocytes.

On transplanting the cells of the bone marrow to the epithelial stroma of the thymus no formation of lymphoid structures was noted. At the same time it is known that when treating irradiated animals with a mixture of bone-marrow cells and lymphocytes from the peripheral lymph nodes which are distinguished by the chromosome marker, regeneration of the lymphoid tissue of the thymus occurs as the result of the bone-marrow cells [4].

Our results indicate that the epithelial stroma of the thymus has a histogenetic effect on the lymphoid tissue, maintaining differentiation of the lymphocytes. The specificity of this effect is indicated also by the results of transferring the cells of the bone marrow to the stroma of the thymus (see table, series VI) and by the absence of differentiation of lymphocytes in chambers in which not thymus but embryonic tissue was placed in the first injection and lymphocytes in the second [3]. We can only make assumptions concerning the nature of the histogenetic effect of the epithelial stroma of the thymus. According to some authors [5] it has a humoral character and stimulates the proliferation of lymphocytes, inhibiting in this case the process of antibody formation.

The effect of the epithelium of the thymus maintaining the histogenesis of lymphocytes is a particular case of those histogenetic interrelationships which exist between the stroma and parenchyma and, apparently, regulate the histogenesis of hematopoietic cells.

SUMMARY

In cultivation of the thymus of newly born mice in a diffusion chamber in vivo there occurs quick disappearance of lymphocytes and proliferation of epithelial membranes with focal structures after the type of Hassal's corpuscles.

Upon repeated injection of lymphocytes from various sources into a chamber containing such epithelial stroma their differentiation is maintained, and there are formed lymphoid accumulations which are often associated with Hassal's bodylike structures. The thymus epithelium in the chamber, however, does not influence the differentiation of bone marrow cells.

In cultivation of the spleen in a diffusion chamber the myeloid cells are retained during the first ten days as foci of 3-5 cells lying on one reticular cell.

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