CULTIVATION OF THE THYMUS OF NEWBORN MICE IN A DIFFUSION CHAMBER

(UDC 612.438:612.646-085.23-019)

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(Presented by Acting Member of Academy of Medical Sciences, USSR G. V. Vygodchikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 4,
pp. 88-92, April, 1966
Original article submitted September 16, 1964

It is known that the thymus plays a significant part in the production of immunity [9, 10]. Thus, in animals thymectomized within a few hours after birth, the lymphoid tissue remains strongly underdeveloped, blood and lymphoid organs contain practically no lesser lymphocytes, and the ability of such animals to reject homografts and to produce antibodies is greatly reduced [5, 6, 9, 10]. When the thymus is transplanted into thymectomized animals, their normal morphology and the immunological activity of their lymphoid tissue become restored [9]. Two hypotheses have been proposed in order to explain this phenomenon. The basis for one of them is the phenomenon of repopulation of other lymphoid organs by competent lymphocytes of the thymus, [4, 9] while the other presupposes a humoral effect of the thymus on the developing lymphoid organs [8].

The stimulation of multiplication of lymphocytes and of antibody formation following injection of thymus extracts favors the second hypothesis [7].

Moreover, convincing data have been obtained on a partial restoration of lymphoid tissue and of immunological activity in thymectomized animals which had been implanted with the thymus in a diffusion chamber [11]. Transplantation of the thymus in a diffusion chamber precludes the possibility of repopulation with lymphocytes or with other cells, but is conducive to the penetration of humoral substances.

The aim of the present investigation was to study the morphology of the thymus transplanted in a diffusion chamber. It was important to find out how the cells of the thymus cultivated in a diffusion chamber differentiate, and what is the "fate" of the epithelial and the lymphoid components of the thymus under such conditions. This information would be important in tracing the origins of those humoral factors by means of which the thymus regulates the histogenesis of the lymphoid tissue, and in studying the mechanism of regeneration of thymus lymphoid tissue during the transplantation of the thymus.

EXPERIMENTAL METHODS

We have used diffusion chambers made of millipore filters type THWP, 25±5 μ thick and having a pore diameter of 0.45 μ. The filters were glued by means of a special adhesive (a solution of a plastic in acetone and chloroform, or MF adhesive) to plastic rings of 14 mm outer diameter and 10 mm inner diameter, and 1.5 mm in height. One of the filters was glued only partially; this was sealed completely only after the tissue was placed in the chamber. The chambers were sterilized for 10 min in 70% alcohol, washed in three changes of distilled water with antibiotics, and dried on gauze pads in sterile Petri dishes.

Thymuses of newborn mice (line C57BL) were used in the transplantation experiments. Either a whole thymus or half a thymus was placed in a chamber. The chambers were sealed and implanted in the peritoneal cavity of adult C57BL mice.

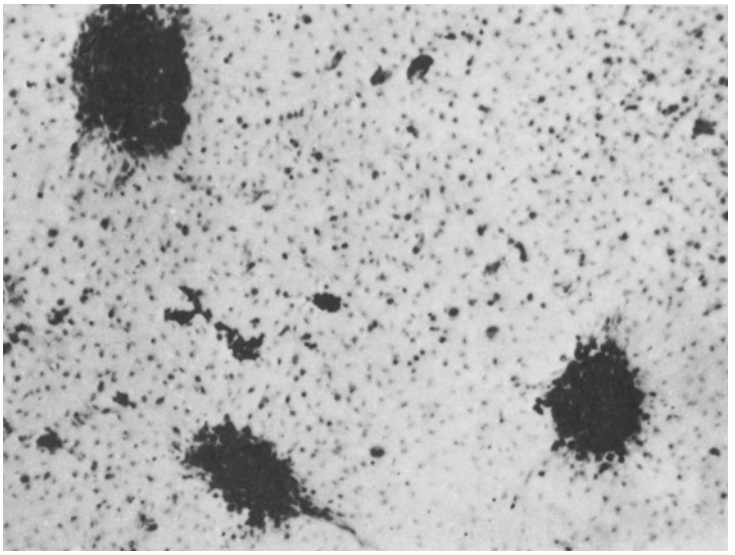

Fig. 1. Culture of thymus of a newborn mouse in a diffusion chamber. Eleven days after transplantation. Whole mount. Fixed in alcohol-formalin, stained with hematoxylin. Obj. 10×.

A total of 14 chambers was fixed and studied. Three chambers were fixed 10 days after transplantation, 5 after 11 days, 2 after 22 days, and 4 after 25 days. The chambers were extracted from the peritoneal cavity, thoroughly freed from the surrounding tissues, opened, and fixed in alcohol-formalin. Whole mounts of filters were stained with hematoxylin or Schiff's reagent and hematoxylin counterstain. The preparations were dehydrated through alcohols, cleared in xylol and mounted in balsam. In some cases tissues were removed from the chambers, embedded in paraffin, and sectioned.

EXPERIMENTAL RESULTS

After 10-11 days of growth of the thymus in a diffusion chamber the entire filter was covered with a dense monolayer of cells. The cell sheet consisted of large rectangular, elongate or polygonal cells with large well outlined nuclei, which contained several nucleoli. The cytoplasm of these cells was often foamy. Among these cells there were encountered some giant cells with 1 or 2 large light-colored nuclei, as well as some degenerating cells with small pycnotic nuclei and with an optically homogeneous cytoplasm.

On all the filters there were small and large cell aggregates which could be easily seen with the naked eye. These aggregates were distributed throughout the entire surface of the filter (Fig. 1). Small cell aggregates contained dozens of cells in several layers. Morphologically such cells were similar to the other cells in the monolayer, i.e., they were also of the epithelial type. In some cells there was a large vacuole in the cytoplasm.

Large cell aggregates consisted of a large number of epithelial cells with small and large nuclei, and sometimes with degenerating nuclei. In these aggregates there was a spherical distribution of cells, in concentric circles around a center. In whole mounts the centers of such aggregates could not be seen because the aggregates were multilayered. The large and the small cell groups gave rise in all directions to a vigorous growth of a single-layered epithelium, but sometimes double cell strands originated in the cell aggregates.

Lymphoid cells were not found on the filters. Sometimes cells with connective tissue-type nuclei were seen.

On two of the whole mounts fixed after 22 days and treated with Schiff's reagent and hematoxylin counterstain, there were small and large cell aggregates connected to each other by strands of cells. The cell aggregates contained in the centers and along their periphery droplets and globules of a Schiff's-positive substance. The same type of secretion was also seen in droplets and globules within cells. Between cell aggregates there were roundish cavities or lacunae lined with epithelial cells, containing some degenerating cells with pycnotic nuclei and pale-staining cytoplasm, as well as small droplets of the secretion.

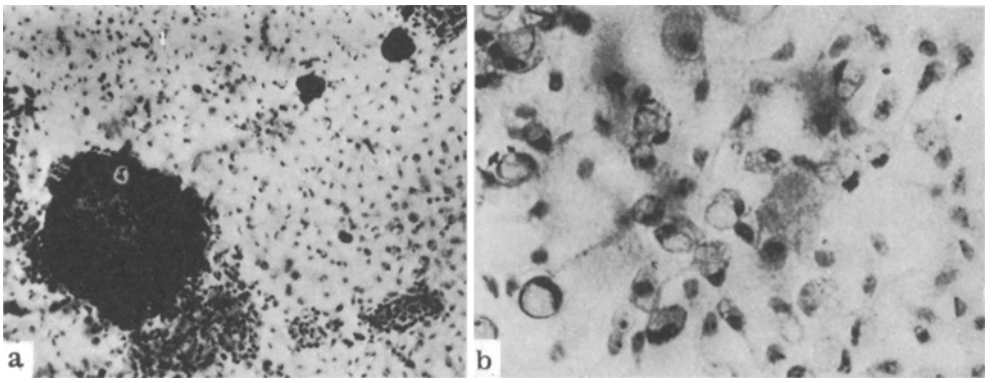

Fig. 2. Culture of thymus of a newborn mouse in a diffusion chamber. Twenty-five days after transplantation. Whole mount. Fixed in alcohol-formalin, stained with hemaxotylin. a) Obj. 10x, b) obj. 24x.

In two other whole mounts fixed after 22 days, only cell monolayers were seen. This could have been due to the fact that when the chambers were opened prior to fixation, most of the cells could have remained on one of the filters. The cells in the monolayers were polygonal or elongate, sometimes with outgrowths, with large nuclei containing several nucleoli, with a foamy, and sometimes vacuolated cytoplasm. Most of these cells gave a diffuse Schiff-positive reaction in the cytoplasm, with Schiff-positive droplets or globules. Vacuoles contained 1 or 2 globules, but some vacuoles did not contain a Schiff-positive substance.

On filters fixed 25 days after transplantation and stained with hematoxylin, there were rounded cells with nuclei pushed towards the periphery, and with a few large vesicular vacuoles in most cells. Consecutive stages of rounding and of vacuolization of cells and of displacement and degeneration of nuclei were seen. There were fewer cell aggregates than at earlier stages. In the centers of large aggregates the zone of degeneration of the epithelium could be clearly seen (Fig. 2).

The data obtained show that under conditions of isotransplantation of the thymus in a diffusion chamber, its lymphoid tissue becomes degenerated, while the epithelial stroma grows out. Similar results were obtained when free thymus was transplanted subcutaneously [2], and when it was cultured in vitro [1]. It is also known that any injury of the thymus leads to its accidental involution, i.e., to the destruction of lymphocytes and the retention and outgrowth of the epithelial stroma. In free transplantation and in accidental involution the epithelial stroma eventually becomes filled with lymphocytes, and thus the normal structure of the organ becomes restored.

Under these conditions there can be four possible sources of regeneration of thymocytes: individual remaining lymphoid cells, reticular cells indistinguishable from epithelial cells, epithelial cells of the stroma (cf. the hypothesis on the epithelial origin of thymocytes [3]), and lymphocytes migrating from other parts of the body.

The results obtained by us have shown that when the thymus is transplanted in a diffusion chamber, as opposed to free transplantation, the restoration of lymphoid tissue of this organ does not take place. Transplantation in the chamber differs from free transplantation first of all in that it excludes the possibility of repopulation of the organ by lymphocytes. It is, therefore, natural to suppose that for regeneration of thymocytes a repopulation by extraneous lymphocytes is necessary.

It is also possible that while some of the lymphocytes placed in the diffusion chamber could have been preserved for some time [12], the conditions within the chamber were on the whole unfavorable for the histogenesis of lymphoid tissue. Consequently, the thymocytes were not restored, although the sources for their formation were present within the chamber. We are currently studying this problem.

Under conditions of transplantation in the diffusion chamber the lymphocytes degenerated, and the transplant represented practically only the epithelial stroma, possibly containing a small number of reticulocytes.

Since it was determined that such transplants are able to restore the normal morphology of the lymphoid tissue and immunological competence of thymectomized animals, it may be concluded that the humoral factor is not produced by thymocytes. Nor is it produced by reticulocytes which are retained in negligible numbers. The humoral

factor must be produced by the epithelial stroma of the thymus. Possibly the secretion of this factor was represented by the morphological entities (droplets and globules) described above.

Thus, the thymus is able to exert a humoral effect on the lymphoid tissue of a thymectomized animal, leading to the restoration of immunological competence of the lymphoid tissue. The source of this effect is apparently in the thymus epithelium. It is not to be excluded, however, that in free transplantation of the thymus into thymectomized animals, their immunological competence may be restored through the repopulation of the lymphoid organs of the recipient by thymocytes. By means of cross-transplantation of the thymus, free and in diffusion chambers, it should be possible to determine if the restoration of immunological competence follows the type of the donor or of the recipient.

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