INDUCTION OF OSTEOGENESIS IN LYMPHOID CELL POPULATIONS IN GUINEA PIGS

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If lymphocytes from the thymus, spleen, and peripheral blood are introduced into a diffusion chamber in guinea pigs along with cells of trypsinized transitional epithelium, bone tissue is induced. This shows that these cell populations are a competent system as regards the induction of osteogenesis, i.e., they contain precursor-cells capable of osteogenesis under the influence of an inducing agent. Lymphocytes from lymph glands under the same conditions are incapable of osteogenesis.

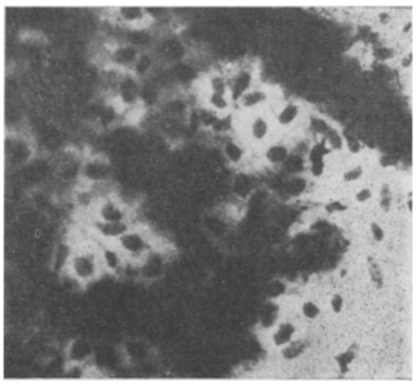

Fig. 1. Diffusion chamber containing thymocytes and transitional epithelium. Total preparation. Foci of osteogenesis on surface of filter; time 33 days. Here and in Fig. 2, stained by Gomori's method and with hematoxylin, 20 ×.

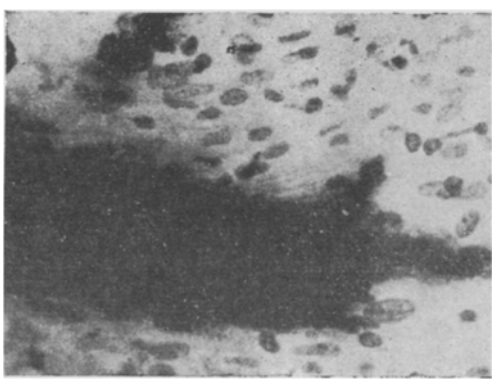

Fig. 2. Diffusion chamber containing splenic lymphocytes and transitional epithelium. Total preparation. Focus of osteogenesis on surface of filter; time, 33 days.

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The use of diffusion chambers, within which osteogenesis is induced by means of transitional epithelium, is a means of studying the properties of cells which are transformed into osteoblasts under the influence of an inducing agent secreted by transitional epithelium [8, 9].

In open systems, i.e., after transplantation of transitional epithelium [3, 4, 7, 11] or ligation of the renal vessels [1, 2] (under conditions when osteogenic activity of transitional epithelium is exhibited), analysis is made difficult because repopulation of cells arriving from elsewhere may take place in the zone of induction. It was previously observed [8, 9] that under diffusion chamber conditions, i.e., in an isolated system, cells of the peritoneal exudate are capable of osteogenesis.

The object of this investigation was to test the ability of peripheral blood cells and cells obtained from the spleen, lymph glands, and thymus, to undergo induction.

EXPERIMENTAL METHOD

Transitional epithelium of the mucous membrane of the urinary bladder was removed from guinea pigs by trypsinization of the stretched mucous membrane at 4° for 12 h or at 37° for 30 min on a magnetic mixer. After trypsinization the cells were centrifuged and suspended in nutrient medium. Circulating blood leukocytes were prepared by the use of heparin and 10% gelatin solution. Blood was obtained by puncture from the heart. Cells of the spleen, lymph glands, and thymus were prepared by grinding the corresponding organ in a mortar in nutrient medium followed by filtration through Kapron. All these cell suspensions were made up in medium No. 199. The diffusion chambers suggested by Algire [10], made from NA millipore filters (pore diameter 0.45 μ), were used. To introduce the cell suspensions into the chamber, a filter of large diameter was laid on an asbestos pad and the cell suspension

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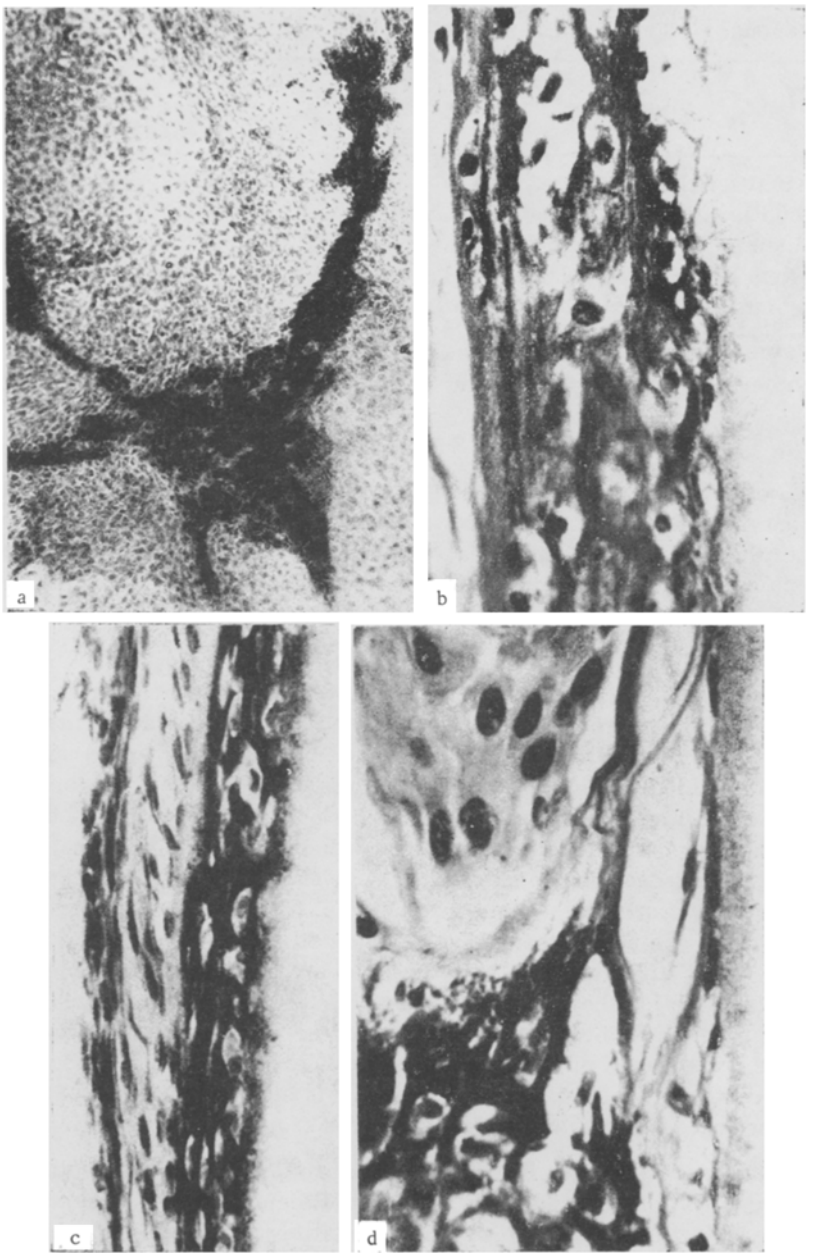

Fig. 3. Foci of osteogenesis in diffusion chambers containing transitional epithelial cells and thymocytes (time 33 days). a) Total preparation. Stained by Gomori's method and hematoxylin, objective 10 ×; b) section. Stained by PAS reaction and hematoxylin, objective 40 ×; c) section. Stained by PAS reaction and hematoxylin, objective 20 ×; d) section. Stained by Gomori's method and hematoxylin, objective 40 ×. Wall of chamber (millipore filter) seen on right of sections.

applied in a volume of 0.1-0.2 ml. After filtration of the liquid through the filter, the latter was covered by a filter of small diameter, and the chamber was greased and implanted in the peritoneal cavity. Epithelium taken from one urinary bladder and 2×10^6 to 5×10^6 lymphocytes of a single category were introduced into one chamber. At different times between the 17th and 33rd days the chamber was freed from surrounding tissue and dismantled, and the filters were fixed with 96° alcohol and treated by the Gomori reaction for alkaline phosphatase and counterstained with hematoxylin. Total preparations were made from most of the filters, but some were cut up into series of sections.

TABLE 1. Induction of Osteogenesis in Diffusion Chambers in the Presence of Transitional Epithelium

Contents of chamber	Time of fixation (in days)	Results*
Transitional epithelium	19-25	0/15
Blood leukocytes + transitional epithelium	19-33	14/19
Spleen cells + transitional epithelium	21-33	7/8
Lymph gland cells + transitional epithelium	31	0/9
Thymus cells + transitional epithelium	33	5/6

*Numerator gives number of chambers containing bone, denominator number of chambers containing living epithelium.

EXPERIMENTAL RESULTS

After trypsinization of the transitional epithelium, the cell suspensions contained a few connective-tissue cells of the tunica propria (as shown by growth of connective tissue in some chambers of the first group, side by side with the formation of epithelial membranes). However, among these cells there were none or too few in which osteogenesis was induced. The osteogenesis taking place in the chambers after addition of spleen or thymus cells or blood leukocytes to the epithelium (Table 1) thus indicates that these populations in fact contain cells capable of responding to induction by osteogenesis.

Morphologically the foci of osteogenesis appeared as typical bone trabeculae, appearing as strongly phosphatase-positive foci. They consisted of osteoblasts and ground substance, in which some of the osteoblasts were embedded. The whole of this structure had the characteristic appearance and stood out against the background of the surrounding tissue, which was phosphatase-negative. The size and shape of the bony foci induced in the chamber differed considerably depending on the localization of the focus on the surface or in the center of the chamber. It is thus still uncertain whether each focus consisted of a cell clone or was formed as the result of aggregation of induced cells. It should be noted that the boundaries of individual foci as a rule were clearly distinguishable. In the size of the foci and the degree of maturity of the bone tissue forming them, the chambers containing thymus cells were clearly distinguishable (Fig. 1, 3). Comparison of the frequency of appearance of bone in the chambers filled with spleen cells (Fig. 2) with blood leukocytes, and thymus cells, showed no difference. In the overwhelming majority of cases among the 2×10^6 to 5×10^6 cells of each of these populations there were sufficient cells capable of osteogenesis under the influence of the transitional epithelium. Meanwhile, cells of lymph glands did not respond by osteogenesis in a single case. Numerous control experiments showed that none of the cell categories described gives rise to bone in a chamber without the action of transitional epithelium.

The four cell populations, consisting basically of the progeny of hematopoietic stem cells and containing peritoneal exudate cells, blood leukocytes, spleen cells and, in particular, thymocytes, thus form a competent system for the induction of osteogenesis by transitional epithelium. Lymph gland cells, on the other hand, do not form a competent system.

In all the cell populations listed above, cells described as immunologically competent are present, for which the agents inducing their differentiation in relation to an immune reaction are antigens. The results described above show that these same populations also contain cells capable of undergoing induction to osteogenesis, i.e., to a completely different type of differentiation through the action of an inducing agent secreted by transitional epithelium [5, 6] and capable of causing the formation of foci of ectopic osteogenesis. Which of the cells in the populations studied are precursors of osteoblasts is not known. It is evident that for the blood leukocytes they are the progeny of hematopoietic stem cells, while for the thymus and spleen they are either lymphocytes (forming the majority in the population), i.e., progeny of hematopoietic stem cells, or reticular cells of the stroma (forming only a very small proportion of the population).

Because of the great ease with which osteogenesis is induced in populations of lymphoid cells by their contact with transitional epithelium, the physiological role of the osteogenic activity of transitional epithelium must be considered or, in other words, how this property is utilized in the living organism where lymphocytes are capable of coming into contact with transitional epithelium if they pass through the blood vessels of the tunica propria of the urinary tract.

Physical contact between the reacting cells and epithelial cells, it will be noted, was not necessary for induction. Exposure to the substance secreted by the transitional epithelium was sufficient [5, 6]. This suggests that a constant, although slow, accumulation of precursor cells takes place in a population of osteogenic cells under the influence of transitional epithelium at the expense of the progeny of hematopoietic stem cells.

If this is in fact true, ectopic osteogenesis under the influence of transitional epithelium reflects one stage in the formation of precursor cells of osteogenic tissue in the body.

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