INDUCTION OF OSTEOGENESIS BY THYMUS CELLS

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Thymus cells of guinea pigs and rabbits have well marked ability to form bone under the influence of transitional epithelium and decalcified bone matrix, respectively. In this property they considerably surpass all other tested populations of lymphocytes and hematopoietic cells. The experiments were carried out in diffusion chambers in which the reacting cells were placed together with the inducers of osteogenesis.

The formation of ectopic bone tissue is an example of an induced disturbance of differentiation in populations of connective-tissue cells. Two inducers of ectopic osteogenesis have received the most study: transitional epithelium of the urinary tract [8, 2-4] and decalcified bone matrix [9, 10]. If these are transplanted, for example, subcutaneously bone tissue formed on account of some of the cells composing the graft bed appears around them. The nature of the inducible osteogenic precursor cells (IOPC) responsible for identification of the inducing stimulus and acting as the source for the ectopic bone cells, is not yet known. In previous investigations the authors have shown [5, 6] that, for the inducer produced by transitional epithelium [3], IOPC exist in several cell populations (blood, peritoneal exudate, and spleen cells) containing cells of thymus origin (T-cells).

The object of the investigation described below was to study the role of the thymus as the source of the IOPC.

EXPERIMENTAL

Experiments were carried out on adult guinea pigs and rabbits.

Transitional epithelium was taken from the wall of the distended urinary bladder after incubation for 12 h in 0.25% trypsin solution at 4°C. The decalcified bone matrix was prepared from the femoral diaphyses [10].

A suspension of thymus cells was prepared by expressing them from the cut organ into medium No. 199 followed by filtration through four layers of Kapron. The cell concentration was adjusted to 10^7/ml.

The inducers and reacting cells were mixed in diffusion chambers [5] made from HA-filters with a pore diameter of 0.45 μ. The required amount of cell suspension was placed on the larger flap of the chamber, lying in a Petri dish, and after the liquid had passed through the filter the inducer, consisting of epithelium taken from one urinary bladder or two or three fragments of decalcified bone matrix, each about 3 mm^2 in area, was added. The second flap with the filter was then applied to the chamber, which was sealed. Allogeneic thymocytes and transitional epithelium or bone matrix were placed together in the chambers, and these were implanted intraperitoneally into allogeneic recipients.

Treatment of the Material. After 25-30 days, the chambers were removed and fixed with 96° alcohol, some as dismantled flaps with the tissue growing on them, the rest whole. Gomori's reaction for alkaline phosphatase or staining with hematoxylin was carried out on the dismantled filters and total preparations were made. The undismantled parts of the chambers and areas of the filters with thick layers of tissue were spread out into sections which were stained with hematoxylin, by the PAS method, and by Gomori's

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TABLE 1. Induction of Bone Tissue in Diffusion Chambers with Thymus Cells

Contents of chambers	Fixation time, days	Chambers with bone / total No.
10^7 guinea pig thymus cells + transitional epithelium	25	5/5
10^7 guinea pig thymus cells + bone matrix . . .	30	0/7
$5 \cdot 10^6$ rabbit thymus cells + transitional epithelium	30	0/8
$5 \cdot 10^6$ rabbit thymus cells + bone matrix.	25	5/5

method. In some cases decalcification with 5% HNO_3 was carried out before the material was spread out into sections.

EXPERIMENTAL RESULTS

Osteogenesis was induced in thymocytes by transitional epithelium but not by decalcified bone matrix (Table 1). Conversely, rabbit thymocytes formed bone under the influence of decalcified bone matrix but not of transitional epithelium. In many of the control chambers (not mentioned in Table 1) in which transitional epithelium, bone matrix, and thymocytes of guinea pigs and rabbits were placed separately, no signs of osteogenesis could be detected.

The transitional epithelium in the chambers grew into membranes consisting of one to three layers of cells. Its structure in the chambers was fully described by the authors earlier [5]. By the time that the chambers were fixed the cells of thymic origin consisted of connective-tissue cells mainly of fibroblast type, with a small number of lymphocytes. In the absence of an induction effect the connective tissue in the chamber gave a negative reaction for alkaline phosphatase by Gomori's method. Chambers filled with bone matrix only contained no cells at the time of fixation but only ground substance of decalcified bone with empty interosseous cavities.

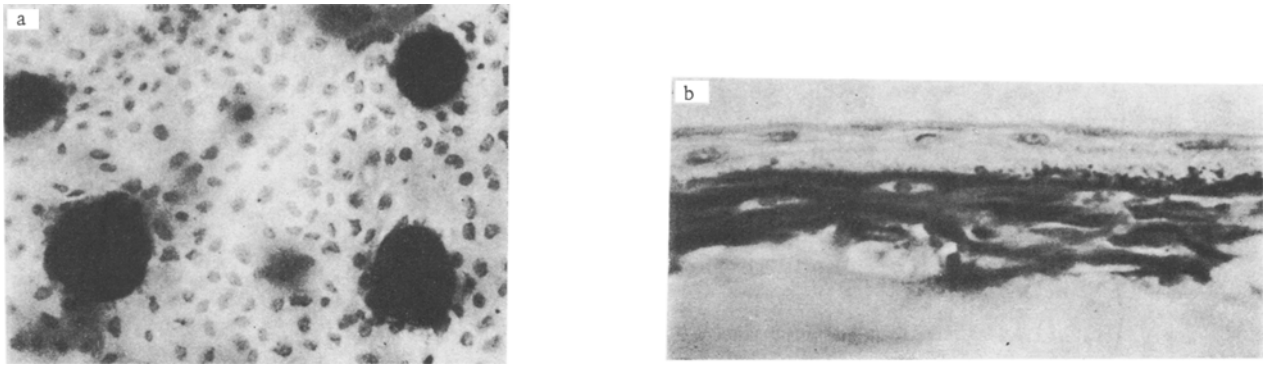

Fig. 1

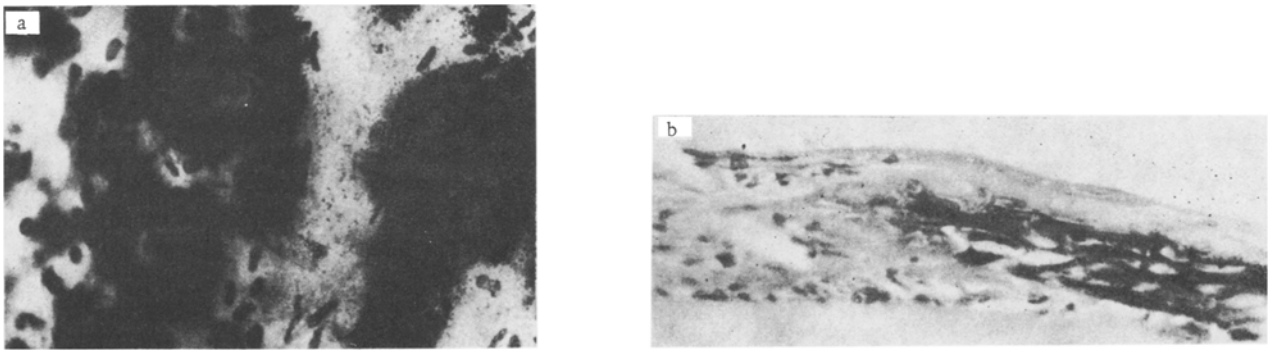

Fig. 2

Fig. 1. Bone tissue induced in diffusion chambers containing transitional epithelium plus guinea-pig thymocytes. a) Total preparations, Gomori's reaction, 20 ×; b) sections, 40 ×.

Fig. 2. Bone tissue induced in diffusion chambers containing decalcified bone matrix plus rabbit thymocytes. a) Total preparations, Gomori's reaction, 20 ×; b) sections, 20 ×.

Foci of bone were found in the connective tissue in the chambers containing transitional epithelium and guinea-pig thymocytes. They consisted of typical bony trabeculae with immured osteocytes and a layer of osteoblasts, and they gave a strong Gomori's reaction. Osteogenic tissue lay on the surface of the filters under the epithelial layers or near epithelial cysts in the center of the chambers. The number of induced foci was difficult to count, for they were apparently confluent (Fig. 1a, b). No cartilage tissue was found in these chambers. Large foci of newly formed bone and cartilage tissue were found in the chambers filled with rabbit thymocytes and bone matrix. The bone tissue had the typical structure and frequently was well calcified. Bone lamellae spread widely over the surface of the filters and the foci of cartilage were situated mainly in the region of resorption of the implanted matrix (Fig. 2a, b).

These results show that osteogenic precursor cells exist among the thymus cells, and that their concentration in the thymocyte population exceeds 10^{-6}. Characteristically the thymus cells were competent in response to those osteogenic stimuli to which the particular species of animal was sensitive in the case of free subcutaneous transplantation of the inducer: osteogenesis by rabbit thymocytes is induced by bone matrix but not by transitional epithelium, while the converse is true of guinea-pig thymocytes.

It is well known that in many cases the thymus is the source of cells required for induction of histogenesis of plasma cells and immune lymphocytes by antigens. Osteogenesis is evidently another type of induced histogenesis for which the competent cells are found in the thymus. The ability of thymus cells to form bone under the influence of an inducer was greater than that of other populations of connective and lymphoid tissue cells. This is shown by comparing the intensity of osteogenesis arising under the influence of transitional epithelium in diffusion chambers containing cells from the thymus, peritoneal exudate, spleen, blood, or lymph glands. In the last case, it must be emphasized, induction of bone has not generally been observed [6].

The question arises whether the thymus is a source of cells required for development, not only of the immune response, but also of ectopic osteogenesis.

It remains to be discovered which cells of the thymus play the role of IOPC. This may be either the thymocytes (progeny of the hematopoietic stem cells) or the stromal precursor cells. Histogenetically the latter are independent of the hematopoietic stem cells [1] and form colonies consisting of clones of fibroblasts [7] (with a cloning efficiency of the order of 10^{-6}), and they belong to the group of easily adhesive cells.

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