PROLIFERATIVE ACTIVITY OF ECTOPIC BONE TISSUE INDUCED BY TRANSITIONAL EPITHELIUM OF THE URINARY BLADDER IN GUINEA PIGS

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Proliferative activity of osteogenic cells of ectopic bone induced by transitional epithelium, and in a stationary state, was studied in guinea pigs by autoradiography. On the side of the bone surface facing the inducing layer and foci of ectopic hematopoiesis, proliferative activity was higher than on the side facing the surrounding connective tissue. Osteoblasts remain only 44 h on the bone surface. Inducing epithelium proliferates more intensively than the noninducing, differentiated epithelium.

The development of ectopic osteogenesis around grafts of transitional epithelium of the urinary bladder is an inductive process. It requires contact between the reacting tissues and the presence of a specific inducing factor, without the action of which this type of histogenesis does not take place [2].

Induced bone is not a self-maintaining system but requires the constant action of the inducer [1, 2]. When absorption of a homograft of transitional epithelium takes place, i.e., when the production of the osteogenetic factor ceases, the induced bone tissue around the grafts is also absorbed [2, 3, 4].

It was therefore decided to study induced ectopic bone in a stationary state and exposed to the action of an inducer. Attention was concentrated on the proliferative activity of the cells in such a system.

EXPERIMENTAL METHOD

Part of the wall of the urinary bladder, cut into pieces with scissors, was autografted beneath the fascia of the rectus abdominis muscle in guinea pigs weighing 250-300 g.

On the 30th day after grafting the animals were given an intraperitoneal injection of thymidine-H^3 in a dose of 1 μCi/g body weight. The animals were killed 2, 18, and 44 h after injection of the isotope. The grafts and surrounding tissue were fixed by Carnoy's method and decalcified with 5% HNO_3 solution for 24 h. After histological treatment serial sections were cut to a thickness of 5-7 μ, and each 10th section was fixed to a slide. The specimens were treated with 3% perchloric acid and then dried and covered with type M liquid emulsion and exposed for 21 days. After development, the specimens were stained with hematoxylin and methyl green–pyronine.

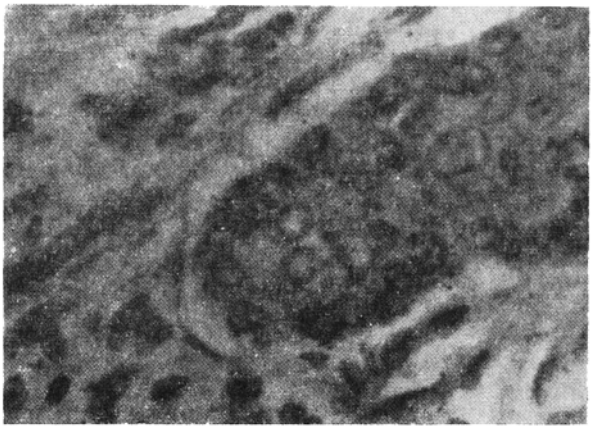

Fig. 1. Distribution of labeled cells in undifferentiated epithelium.

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 12, pp. 79-82, December, 1970. Original article submitted June 11, 1970.

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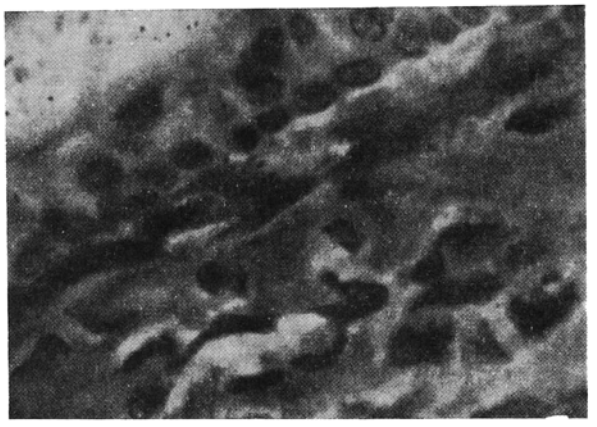

Fig. 2. Region of bone adjacent to inducing epithelium.

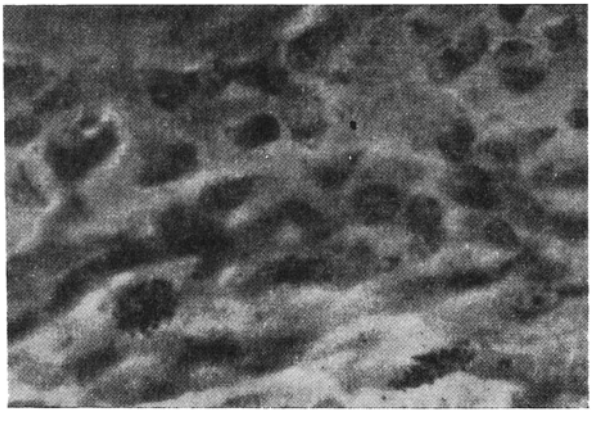

Fig. 3. Region of bone lying on side of connective tissue, i.e., not in contact with epithelium.

Cells with five or more grains above the nucleus were regarded as labeled. At each stage of the experiment two grafts were studied. During analysis of the labeling the transitional epithelium in the grafts was divided into two regions.

1. Differentiated epithelium, characterized by vertical anisomorphism. This layer consists of basal, intermediate, and superficial layers of epithelial cells. This type of epithelium always has a basement membrane. No osteogenesis is found in the region of this epithelium.

2. Undifferentiated epithelium, consisting of 2-5 layers of elongated cells (Fig. 1). This epithelium had no basement membrane. It is inductive, since the bone is in contact with epithelium of this type. To study the proliferative activity of the osteogenic cells, the bone was divided into two regions: 1) a region of bone adjacent to the inducing epithelium (Fig. 2), and 2) a region of bone on the opposite side of the focus of induced bone, i.e., not facing the epithelium (Fig. 3). The percentage of labeled cells was calculated separately for these two regions. In the osteogenic tissue the percentage of labeled cells was calculated separately for osteoblasts and preosteoblasts. The percentage of labeled cells was also calculated separately in the zone of induction, where foci of hematopoiesis were present. At each stage of the investigation 2000 cells were counted.

EXPERIMENTAL RESULTS

The experimental results are shown in Table 1. The percentage of labeled cells in the undifferentiated epithelium was 0.6 at all times (2, 18, and 44 h) after injection of thymidine-H^3. In places where the undifferentiated epithelium was in contact with bone tissue, the layer of outer osteoblasts lay directly under the epithelium. In some places epithelial cells protruded into the layer of osteoblasts and proosteoblasts.

The percentage of labeled cells in the differentiated epithelium reached 0.05 at all times (2, 18, and 44 h) after injection of thymidine-H^3. No foci of osteogenesis could be found in the region of this epithelium.

Labeled cells in both differentiated and undifferentiated epithelium were found mainly in the basal layer. The percentage of labeled preosteoblasts on the side of the bone bordering the epithelium was 5 at all times after injection of thymidine-H^3. The proportion of labeled osteoblasts was 2%.

The percentage of labeled osteoblasts and preosteoblasts on the opposite side, i.e., on the side bordering the connective tissue, was 1 and 3 respectively.

The percentage of labeled osteoblasts and preosteoblasts in the region of the bone surface near a focus of hematopoiesis was 2.5 and 5.5 respectively. Numerous young osteogenic cells (spindle cells or cells resembling fibroblasts) were found in this region. These cells were immediately next to the bone surface. Labeled lymphocytes were also present in this region, at all times (2, 18, and 44 h) after injection of thymidine-H^3.

Solitary labeled osteocytes, immured in the ground substance, could be seen 44 h after injection of thymidine-H^3.

TABLE 1. Percentage of Labeled Cells in Epithelium of Induced Bone

Time after injection of isotope (in h)	Noninducing epithelium	Inducing epithelium	Region of bone bordering epithelium		Region of bone facing surrounding connective tissue		Region of bone facing foci of hematopoiesis	
			osteoblasts	preosteoblasts	osteoblasts	preosteoblasts	osteoblasts	preosteoblasts
2	0.05	0.6	2	5	1	3	2.5	5.5
18	0.05	0.6	2	5	1	3	2.5	5.5
44	0.05	0.6	2	5	1	3	2.5	5.5

The distribution of labeled osteoblasts and preosteoblasts was irregular over the whole surface of the bone. The percentage of labeled cells varied from 2 to 7 in the case of preosteoblasts and from 0.5 to 4 for osteoblasts.

This study of ectopic osteogenesis in a stationary state showed that the undifferentiated epithelium retains greater proliferative activity than the differentiated epithelium at all times after injection of thymidine-H^3. Since bone is formed only near the undifferentiated epithelium, and no osteogenesis takes place near the differentiated epithelium, it can be assumed that the inductive activity of the epithelium is associated with its proliferative activity.

Bone tissue induced by transitional epithelium proliferates over the whole surface, but in regions facing the inducing layer of transitional epithelium the mitotic activity of the preosteoblasts and osteoblasts is higher than in regions where bone is not in contact with epithelium.

The proliferative activity of the osteogenic cells is also high in the region where foci of hematopoiesis are in contact with the bone surface. Here numerous young osteogenic cells can be seen. This perhaps indicates that transformation of osteogenic precursor cells takes place in the immediate vicinity of the bone surface.

Since no labeled osteocytes could be found 18 h after injection of thymidine-H^3, but they were present 44 h after injection, it is evident that osteoblasts remain on the surface of the bone for more than 18 h but less than 44 h.

Labeled osteoblasts and preosteoblasts are not uniformly distributed on the bone surface, but in colonies, presumably indicating partial synchronization of the process of osteogenesis.

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