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Short Communications

The Role of Host Site in Bone Induction by Transplanted Xenogenic Epithelial Cells

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Human epithelial tissue culture line cells ("K" line) were injected into the leg or abdominal wall muscle, or subcutaneously, in cortisone-conditioned rats. Cartilage and/or bone was induced readily in either of the muscular sites, but not subcutaneously.

Key words: Transplantation — Bone — Induction — Osteogenesis — Amniotic cells.

Des cellules épithéliales humaines, obtenues par culture de tissu (de lignée "K"), sont njectées dans les muscles de la cuisse et de la paroi abdominale ou dans le tissu sous-cutané de rats conditionnés par la cortisone. Du cartilage et/ou de l'os se forment dans le tissu musculaire, mais non dans le tissu sous-cutané.

Zellen aus der Kultur von menschlichem Epithelialgewebe („K"-Stamm) wurden Cortison-vorbehandelten Ratten in die Bein- oder Bauchwandmuskulatur oder subcutan injiziert. Die Bildung von Knorpel und/oder Knochen wurde in diesen zwei Muskelgeweben leicht ausgelöst; dies war jedoch an den subcutan behandelten Stellen nicht der Fall.

Two important questions relating to bone induction evoked by grafts of xenogenic cell lines (Anderson *et al.*, 1964; Włodarski, 1969; Włodarski *et al.*, 1970, 1971) are still open. These are the nature of the induction and the identity of the potentially osteogenic host cells.

Urist and others (Urist *et al.*, 1967, 1968, 1969; van de Putte and Urist, 1965; Yeomans and Urist, 1967) described a bone induction "principle", a complex of macromolecular protein substances, emanating from the decalcified matrices of cartilage, bone and dentine. The "principle" is acid-resistant, but sensitive to protein-denaturing influences such as hyperthermia, freezing and thawing, X-irradiation, etc.

However, it seems to us that the inductor involved in the osteogenesis produced by epithelial cell grafts must differ from the bone induction principle of Urist. Epithelial cells treated with HCl and lyophilized fail to induce bone (Włodarski, unpublished results). It is necessary that the epithelial cells should be living for bone induction to occur, and the possibility cannot be excluded that the production of the bone induction by them is triggered off by contact with host cells or fluids. The production of induction seems to be independent of the proliferation of grafted cells. This is shown by the fact that WISH or transitional epithelial cells continue to divide following exposure *in vitro* to X-rays (5×10^3 and 120×10^3 R respectively), but bone induction is weakened (Włodarski *et al.*, in preparation).

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According to Urist and others (Büring and Urist, 1967; Urist *et al.*, 1969; Nogami and Urist, 1970) the cells which respond to the bone induction principle of demineralized bone are mesenchymal populations derived from muscle. It has been proposed that bone formation following the grafting of xenogenic epithelial cell lines into leg muscles merely results from a non-specific irritation of periosteum (Friedenstein, 1968), and by Urist that endomysial mesenchymal cells are responsible for the production of induced bone. We have therefore attempted to obtain induction in sites distant from the skeleton.

Materials and Methods

Human amnion cells of the "K" line were used, culture conditions being as described previously (Włodarski *et al.*, 1970). The K cells were suspended in phosphate buffer solution, pH 7.0. For grafting, approximately 3×10^6 cells in 0.2 ml of phosphate buffer solution were injected into the abdominal muscles of rats weighing 48/131 g. To be certain that the cells were injected intramuscularly, a skin incision was made under anaesthesia, the muscle exposed, and the needle point entry made under visual control. The wound was stitched. In some cases, the same number of K cells was injected subcutaneously in the abdominal region, or intramuscularly in the hind leg. All the animals received cortisone acetate (Cortisyl, Roussel) one or two days prior to the injection of the cells. The dose of cortisone, duration of experiments and results are summarized in Table 1. Tissue for histological examination was fixed in Bouin's solution and embedded in wax. Sections were stained by routine methods.

Table 1

Weight of animals (g)	Immunosuppression (days and doses of cortisone acetate injection[a])	Days after grafting K cells	Abdominal wall grafts containing	
			surviving K cells	cartilage and bone
51	−1 (10); 0 (5)	11	+	+[b]
50	−1 (10); 0 (5)	11	−	−[b]
48	−1 (10); 0 (5)	11	+	+[b]
60	−1 (10); 0 (10) + 7 (5)	10	+	+[c]
61	−1 (10); 0 (10) + 7 (5)	10	−	+[c]
60	−1 (10); 0 (10) + 7 (5)	10	+	+[c]
95	−2 (15); 0 (15) + 3 (10)	10	−	+
83	−2 (15); 0 (15) + 3 (10)	10	+	+
131	−2 (15); 0 (15) + 3 (10)	10	−	−

[a] Numbers before parenthesis refers to days (before, at, or after grafting of cortisone injection. Numbers in parenthesis refer to dose of cortisone acetate in milligrams.

[b] Cartilage and bone induction in leg muscle.

[c] Neither cartilage nor bone induction with K cells grafted subcutaneously.

Results and Discussion

When K cells were injected into abdominal (Fig. 1) or leg muscle, they survived and cartilage and bone induction occurred. With subcutaneous injection, although the K cells survived well, no inductions occurred. This latter finding is in agreement with previous work with mice (Włodarski *et al.*, 1971) in which human amniotic cell line WISH failed to induce bone when grafted subcutaneously though they

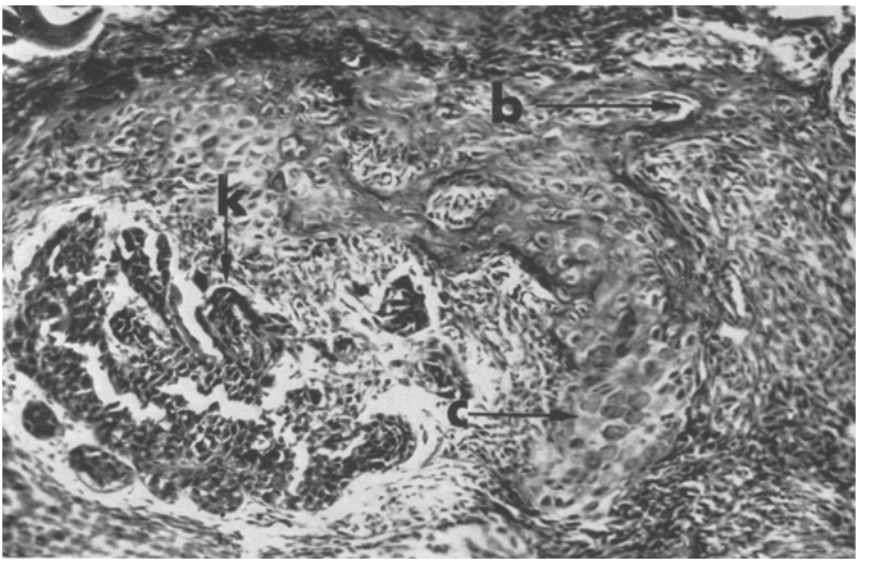

Fig. 1. Section of host abdominal muscle 2 weeks after implantation of K cells. The periphery of the field is occupied by connective tissue and muscle is visible at top left. Towards the centre is a group of darkly-staining, surviving K cells (arrow, *k*). This is almost surrounded by cartilage (*c*) and bone (*b*). Haematoxylin and eosin. ×250

did so intramuscularly. In mice, in the present experiments, no bone was induced by K cells grafted to, and surviving in, the smooth muscle of the urinary bladder wall. Stanbridge and Perkins (1969) and Curtis and Perkins (1971) grafted human epithelial cell lines subcutaneously into mice treated with mouse antilymphocytic serum. Though the cells survived, neither bone nor cartilage formation was reported. Cells of the same kind when grafted intramuscularly within mice treated with cortisone (Włodarski, 1969), or with mouse antilymphocytic serum, (Włodarski and Hancox, in preparation) provoked cartilage and bone formation.

These results indicate that the injection site influences hard tissue induction and point to a target within skeletal muscle. The formation of bone around K cells grafted to abdominal muscle shows that periosteal activity can be ruled out as essential in xenogenic cell bone induction. They also show that K cells can be added to the list of established epithelial cell lines possessing osteoinductive properties.

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