EFFECTS OF DIFFERENT CONCENTRATIONS OF SERUM ON CARTILAGE GROWTH IN AN ORGAN CULTURE SYSTEM

R. SHURTZ-SWIRSKI, D. LEWINSON, P. SHENZER, AND M. SILBERMANN

Laboratory for Musculoskeletal Research, The Rappaport Institute for Research in the Medical Sciences, Faculty of Medicine—Technion, P. O. Box 9649, Haifa 31096 Israel

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SUMMARY

The purpose of the present study was to examine the effects of various concentrations of serum on the behavior of neonatal condylar cartilage when cultured in an organ culture system. Mandibular condylar cartilages were obtained from newborn ICR mice, of which the zone of undifferentiated chondroprogenitor cells along with a few layers of young cartilage cells were cultivated at the medium-air interface. The incubation medium included fetal bovine serum at concentrations ranging from 0 to 10%, and the explants were kept in vitro up to 10 d. The serum-free medium maintained the chondrogenic expression, and the overall size of the cartilagenous portion of the explants increased with the decrease of the concentrations of serum in the medium. When explants were labeled with [3H]thymidine and were then processed for autoradiography, the peak of labeling was noticed at 48 h, a feature that recapitulated itself in all cultures (73, 140, 175, 201, and 129 labeled cells per chondroprogenitor zone in explants grown in 0, 1, 2.5, 5, and 10%, respectively). It can be concluded that serum-free medium maintains the chondrogenic phenotype of condylar cartilage in vitro.

Key words: cartilage; organ culture; serum-free medium; chondrogenesis.

INTRODUCTION

Cartilage explants from a variety of animal sources were studied in vitro as organ cultures (Silbermann and Maor, 1984). Culture media have been traditionally supplemented with various factors to maintain tissue viability and promote optimal growth. Such supplements included chicken embryo extracts or sera or both from different sources. It has been however realized however that the latter included significant levels of hormones or growth factors or both that could affect tissue development and its phenotypic expression (Elmer, 1983).

The mandibular condylar cartilage has been used in vitro for developmental studies (Hall, 1978; Stutzmann and Petrovic, 1982), and more recently it has been recommended as an in vitro model for bone formation (Silbermann et al., 1983, 1987a, b). It became evident that precursor cells in this organ (the chondroprogenitor cells), that differentiate into chondroblasts in vivo, shift their developmental pattern in vitro and instead of following the chondrogenic lineage obtain osteogenic characteristics (Silbermann et al., 1987a). That is, under different epigenetic stimuli they differentiate into osteoblast-like cells and consequently form chondroid bone (Silbermann et al., 1987a).

To further elucidate the effect of serum on the behavior of this organ in vitro, this study utilized various concentrations of serum and followed their effect on this tissue. Our present findings were mainly based on quantitative morphologic data that were obtained at the light microscopy level. It could be demonstrated that serum serves as a dominant factor in regulating the developmental pattern of the cultured tissues, as noticed by the response of the tissues as a whole and their young progenitor cells.

MATERIALS AND METHODS

Culture System

ICR mice, 1- to 2-d old, were anesthetized with ether. Using a surgical microscope the mandibular condyles were aseptically dissected away from the mandibles and cleaned of all soft tissues and of the underlying bone, leaving only the zone of chondroprogenitor cells along with a few layers of young chondroblasts. Tissues were collected in ice-cold Hanks' balanced salt solution, and the separated tissues were transferred onto 0.45-μm millipore filters (Millipore, Bedford, MA) cemented onto stainless steel grids (Michigan Dynamics, Garden City, MI) at the interface of air and medium. The medium composed of BGJb medium (Fitton-Jackson modification, Biological
Industries Beit HaEmek, Israel) containing fetal bovine serum (FBS) at concentrations ranging from 0 to 10%, ascorbic acid (300 μg/ml), and glutamine (200 μg/ml). Cultures were carried out for as long as 10 d in a humidified incubator at 37°C in an atmosphere of 5% CO2:95% air. The medium was changed every 48 h. Two microcurie per milliliter [3H]thymidine (5 Ci/mmol; Amersham Radiochemical Centre, Amersham, UK) were added to the medium for the last 18 h of the culture.

**Light Microscopy**

Tissues that were designated for structural studies were initially washed in Hanks' salt solution and were subsequently fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 90 min., postfixed in 1% OsO4-1.5% Fe(CN)6 for 60 min, dehydrated, and embedded in Epon 812. Thick sections (1 μm) were stained with 1% toluidine blue and were used for morphometric analysis using the Cue-2 computerized image analysis system. The measurements included the explants' overall width and length as well as the length of the cartilage proper. The latter constituted the summation of the diameter of the chondroblastic and hypertrophic layers. The means were based on at least nine measurements, obtained from three different cultures.

**[3H]-Thymidine Autoradiography**

Labeled condyles were washed, fixed in glutaraldehyde, and embedded in Epon. Sections (1-μm thick) were mounted on glass slides, coated with nuclear track emulsion (NTB-2, Kodak, Rochester, NY), and placed in light-tight black boxes. After 3 to 4 wk of exposure at 4°C, autoradiographs were developed in D-19 developer (Kodak) at 18°C, fixed, and lightly stained with toluidine blue.

**Fig. 2.** [3H]Thymidine autoradiographs of explants after 48 h in culture at various concentrations of serum. It can be seen that the isotope was incorporated by cells within the chondroprogenitor zone. Note the growth of the explant at all serum concentrations as well as the preservation of the various cellular zones. a, Serum-free culture; b, culture supplemented with 1% FBS; c, culture supplemented with 2.5% FBS; d, culture supplemented with 10% FBS. Toluidine blue, ×96.

**Fig. 3.** Section through an explant after 10 d incubation in serum-free medium. Note the cartilaginous appearance throughout the explant. a, General appearance. ×96. b, Higher magnification of (a) showing a well-preserved perichondrium (P) and an intact cartilage (C). Toluidine blue, ×576.