PRODUCTION OF ALBUMIN AND \( \alpha \)-FETOPROTEIN IN PRIMARY CULTURE OF FETAL HUMAN LIVER CELLS ON COLLAGENOUS SUBSTRATA IN THE PRESENCE OF HYDROCORTISONE

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SUMMARY

When primary cultures of fetal human liver cells established on type I collagen gels were compared to sister cultures developed on tissue culture plastic, the cells in contact with type I collagen secreted albumin at a higher rate than those without contact. The albumin secretion was dependent on the presence of hydrocortisone (HC) in the medium. Also, \( \alpha \)-fetoprotein (AFP), of which the level decreased gradually and became undetectable after 6 d regardless of the presence or absence of HC in the cells cultured on plastic, was maintained for longer periods of time by plating the cells on type I collagen gels in the presence of HC. Different secretion rates of albumin and AFP were observed after Day 13 and Day 16, respectively, between cells maintained on type I collagen gels and those on film plastic. The cells secreted larger amounts of both albumin and AFP in plates coated with type IV or I collagens than with fibronectin after Day 10. The cells cultured on type I collagen gels were cuboidal in shape, whereas those on plastic were flattened in cultures with HC. These data indicate that the secretion of human albumin and AFP is facilitated by synergies between HC and collagenous substrata.

Key words: human liver; culture; collagen; albumin; \( \alpha \)-fetoprotein.

INTRODUCTION

The study of gene expression of \( \alpha \)-fetoprotein (AFP) and albumin may reveal the control mechanism involved in the embryonic development and hepatocarcinogenesis (3,16,18). Glucocorticoids have been shown to modulate the expression of these proteins (1,2,5-7,15). Cellular differentiation or growth in vitro has been shown to be influenced by placing cells on a variety of forms of extracellular matrix. These results have been obtained in studies that were carried out with primary cultures of mouse mammary epithelial cells, rat liver cells, and others (4,8,13). The present study describes primary cultures of fetal human liver cells, of which the maintenance of both AFP and albumin is dependent on the collagenous substrata and hydrocortisone (HC).

MATERIALS AND METHODS

Preparation of collage gels. A collagen solution was prepared from rat tails essentially as described by Michalopoulos and Pitot (13). Collagen gels were prepared by spreading 0.2 ml of the collagen solution (1 mg/ml) in a 35-mm dish (Falcon) followed by the addition of 0.2 ml of 2x concentrated medium and 0.34 N NaOH. Collagen films were prepared by introducing 0.2 ml of the collagen solution into a 35-mm dish followed by drying at room temperature for 20 min. For preparing gelatin plates, the 35-mm dishes received 1 mg/ml gelatin (Difco, Detroit, MI) in phosphate buffered saline (PBS) at 4°C over-night, were aspirated, and used immediately. In the experiments using 24-multiwell cluster dishes (Falcon), the dishes were coated with 10 \( \mu \)g/ml of fibronectin (Sigma, St. Louis, MO), type I or IV collagen (Nitta-gelatin Co., Osaka, Japan) in 0.5 ml of PBS, kept at 37°C for 1 h, aspirated, and used immediately.

Primary cell culture. Specimens of human fetal liver were obtained at legal abortions, performed on sociomedical indications. The fetal age ranged from 16 to 23 wk of gestation. Livers were minced and digested with 1000 \( \mu \)g/ml dispase (Godo-shusei Co., Chiba, Japan) in Hanks’ solution for 20 min at 37°C. The cell suspensions were filtered through stainless steel mesh and centrifuged 3 times at 50 Xg for 5 min. The pellets were resuspended in a growth medium composed of RPMI 1640 and 5% bovine serum, to which were added 5 \( \mu \)g/ml insulin, 0.1% lactalbumin hydrolysate, and 60 \( \mu \)g/ml kanamycin. Primary culturing was carried out at 37°C in a humidified atmosphere with 5% CO\(_2\). Cell suspensions (1.3 ml) were inoculated into 35-mm (3 \( \times \) 10\(^6\) cells/dish) and 24 multiwell cluster dishes (Falcon, 7 \( \times \) 10\(^6\) cells/well), respectively. Two cultures were initiated for each experiment. On Day 2, the bovine serum was...
Fig. 1. Effect of HC and collagen gels on the secretion of albumin and AFP. Liver cells (3 × 10⁶ cells/35-mm dish) were inoculated on dishes coated with (a) or without (b) type I collagen gels (1 mg/ml). After 2 d of inoculation, cells were cultured in serum-supplemented medium with or without HC (5 × 10⁻⁷ M). Albumin and AFP in culture fluids were determined by ELISA. (●-●) Data were corrected for carryover of albumin from one measurement to the next in collagen gel cultures. Insert shows the content of albumin andAFP per 10⁶ cells per 48 h. Values are the mean ± SD of two replicates from two separate experiments.