HYDROCORTISONE STIMULATION OF HUMAN MAMMARY EPITHELIAL CELLS

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SUMMARY

Rapid proliferation of mammary epithelial cells derived from biopsy specimens of human fibroadenomas was observed when medium was supplemented with ten percent fetal bovine serum and hydrocortisone (5 μg per ml⁻¹). Hydrocortisone in combination with FBS also led to a 2.5-fold increase in cell cluster attachment and subsequent colony formation. A similar effect was not observed with human serum. In contrast to fibroblast cell systems, insulin did not significantly alter cell growth. The results show that a mitogenic response to glucocorticoids by mammary epithelium may depend on the presence of factors in sera.

Key words: mammary; serum; hydrocortisone.

INTRODUCTION

Clusters of cells, routinely obtained by mechanical disruption of human mammary fibroadenoma samples, form colonies of epithelium capable of replication in culture for approximately 3 weeks (1). These cells resemble in morphology and growth pattern those isolated from lacteal secretions (2) and reduction mammoplasty tissues (3). A similar cell type also has been observed in heterogeneous cultures established from samples of ductal carcinoma (4). A recent report emphasized that the absence of fibroblast contamination coupled with the ease of storage at low temperature permits the use of cultures from fibroadenomas for the study of extracellular factors that regulate the growth of mammary cells (1). The current study examined the effects of different sera and hormones on the attachment and proliferation of these cells.

MATERIALS AND METHODS

Biopsy samples of human mammary fibroadenomas were placed in medium 199 supplemented with one percent fetal bovine serum and gentamicin (50 μg per ml) and delivered to the laboratory at ambient temperature. The tissues were either processed immediately or stored overnight at 4⁰C. No obvious changes in culture establishment and proliferation were noted with sample storage. Specimens for this study were obtained from 12 patients ranging in age from 18 to 60 years.

Suspensions of single cells and cell clusters were obtained by cutting and scraping tumor specimens with a scalpel. Cells were washed in medium and cultures were established by inoculating a suspension containing single cells and 50 to 250 cell clusters into 35-mm plastic dishes in medium 199 supplemented with serum. Sera used included one batch of virus-tested mycoplasma-free fetal bovine serum (Flow Laboratories, Rockville, Md.) and/or three batches of human serum negative for hepatitis-associated antigen (Grand Island Biological Co., Grand Island, N.Y.)

RESULTS

The initial series of experiments was designed to reduce the concentrations of each serum to the lowest levels that permitted maximum cell growth. Equal numbers of cell clusters were inoculated to dishes containing medium with FBS or human serum in concentrations from 0.1 to 30.0%. Cultures were fluid-changed at 2 days and the experiments terminated at 7 days. Cell colony number and size were the most obvious cultural characteristics by which cell survival was measured. The greatest number of colonies were formed with both sera at a concentration of 10%; cultures grown in FBS contained approximately 2.5 times more colonies than those in human serum (Fig. 1). A slight reduction in the number of colonies was noted with concentrations of

1Supported in part by NCI Contract CB-33898.
Fig. 1. Colony formation by clusters of human mammary cells derived from a biopsy specimen of fibroadenoma. Cultures established in 35-mm tissue-culture dishes in medium 199 supplemented with increasing amounts of fetal bovine serum (O--O) or normal human serum (O---O). Culture fluids were renewed on day 2 and the experiment terminated on day 7. Results are expressed as the average number of colonies for triplicate cultures; vertical bars indicate range among samples.

Colonies arose from small clusters containing 10 to 30 cells rather than from single cells. Large cell clusters rarely attached and were removed from the cultures at first fluid change. Cell suspensions also were seeded in medium alone or in medium supplemented with 10% FBS. Colonies failed to originate from two tumors in the absence of serum whereas two additional samples gave rise to approximately 15% the number of colonies observed with FBS.

The replication of mammary epithelial cells then was studied in the presence of hormones shown to be mitogenic in fibroblast systems (5, 6). Cells were inoculated to medium containing insulin (5 µg per ml⁻¹) and/or hydrocortisone (5 µg per ml⁻¹) as well as 10% FBS and 10% human serum singly or in combination. Culture fluids were renewed on days 2 and 9. The experiments were ended on day 10 following 24 hr incubation in medium containing 1 µCi per ml⁻¹ [³H]thymidine (2.2 Ci per mmol⁻¹).

Fig. 2 expresses the average number of colonies from observations on triplicate cultures. The data illustrate that hydrocortisone enhanced colony number approximately 2.5 times above the control in the presence of FBS or FBS in combination with human serum. In contrast, insulin alone appeared to have no significant effect. Larger colonies were noted in cultures with FBS, and colony size was further increased by hydrocortisone singly or in combination with insulin. Fig. 3 gives the results from counts on the number of labeled cells in cultures. In contrast to the previous observation that FBS promoted colony number, a greater number of colonies was observed in cultures with 10% FBS or FBS in combination with human serum compared to cultures with 10% human serum alone.