THE INFLUENCE OF CELL SHAPE ON THE INDUCTION OF FUNCTIONAL DIFFERENTIATION IN MOUSE MAMMARY CELLS IN VITRO

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SUMMARY

To define more clearly the in vitro conditions permissive for hormonal induction of functional differentiation, we cultured dissociated normal mammary cells from prelactating mice in or on a variety of substrates. Cultivation of an enriched epithelial cell population in association with living adult mammary stroma in the presence of lactogenic hormones resulted in both morphological and biochemical differentiation. This differentiation, however, was not enhanced over that seen when the cells were associated with killed stroma, provided that the killed stroma had a flexibility similar to that of the living stroma. Cells cultured in inflexible killed stroma usually did not differentiate. Cells cultured within the flexible environment of a collagen gel, but removed from the gas-medium interface, differentiated in a manner similar to those cultured in flexible stroma. Cells cultured on the surface of an attached collagen gel were squamous, and their basolateral surfaces were sequestered from the medium; they did not differentiate. Cells cultured on floating collagen gels were cuboidal-columnar, with basolateral surfaces exposed to the medium, and showed good functional differentiation. Cells cultured on inflexible floating collagen gels were extremely flattened and had exposed basolateral surfaces, and showed no evidence of functional differentiation. We infer that assumption of cuboidal to columnar shapes similar to those of mammary cells in vivo may be important to the induction of functional differentiation in vitro. The additional requirement of basolateral cell surface exposure also is important.

Key words: Cell differentiation; mammary epithelium; cell shape; cell substrate.

INTRODUCTION

Synthesis and secretion of milk by mouse mammary epithelial cells in vivo involves interplay among a complex of hormones (1,2). Cultivation in vitro of explanted whole or fragmented mammary glands from midpregnant mice in defined medium containing lactogenic hormones results in the induction of morphological and biochemical markers characteristic of functional differentiation (see 2, 3 for review).

Attempts to maintain or induce the differentiated state in dissociated mammary cells grown on glass or plastic substrates in the presence of lactogenic hormones have met with little success (4–7). In contrast, Emerman and coworkers (8,9) have demonstrated that dissociated mouse mammary cells show hormone-specific induction and maintenance of morphological and biochemical differentiation when cultured on floating collagen gels, but not when cultured on plastic or on collagen gels left attached to the culture dish.

The floating collagen gel provides an environment different from that of the plastic culture dish in several ways: (a) epithelial cells can interact with stromal elements; (b) the cells are closer to the gas-medium interface; (c) basolateral cell surfaces have access to nutrients and hormones in the

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medium; and (d) the flexibility of the collagen substrate allows the cells to change shape from squamous to cuboidal-columnar (8-10). Recent work by Emerman et al. (11) has indicated that no one of these features is solely responsible for the functional differentiation that occurs when all are present. Of particular significance is the suggestion that cell shape change may have a major influence on the ability of mammary epithelial cells to differentiate functionally in vitro; recent reports by other investigators indicate that cell shape is important in regulation of proliferation (12) and differentiation (13) of other cell types.

In the experiments reported here we have attempted to define more clearly the factors affecting secretory differentiation of mouse mammary cells in vitro. The effect of oxygen accessibility was investigated by culturing mammary cells far from the gas-medium interface, while still maintaining basolateral surface exposure, compact cell shape, and the opportunity for epithelial-stromal interactions. The effect of substrate flexibility was investigated by culturing mammary cells on a variety of flexible or resilient collagen-containing substrates, while basolateral surface exposure and oxygen accessibility were optimized. We will show that substrate flexibility, permitting the assumption of a cuboidal-columnar shape, is critically important for the induction of functional differentiation in these cells. The contributions of basolateral cell surface accessibility and epithelial-stromal interactions will be discussed.

**MATERIALS AND METHODS**

**Dissociation procedure.** Mammary glands from 10 to 12-d-pregnant BALB/cCrl or BALB/cfC3H/Crl mice were dissociated in a solution of 250 U/ml collagenase (Type I, 180-245 U/mg; Sigma Chemical Co., St. Louis, MO) and 4% bovine serum albumin (BSA, Fraction V; Sigma) in Hanks' balanced salt solution (HBSS) for 1 h at 37°C (14). The epithelial population was enriched by centrifuging for 3 min at 800 rpm, then resuspended in Medium 199 containing 0.05% pronase (B grade, 45,000 U/g; Calbiochem-Behring Corp., San Diego, Ca) for 45 min. After pelleting, the cells were resuspended in Medium 199 containing 10% bovine serum and passed through 10 μm mesh Nitex cloth (Tetko, Inc., Elmsford, NY). Cell viability was determined by trypan blue dye exclusion.

**Preparation of collagen gels and culture procedures.** A stock solution of rat-tail tendon collagen in 0.1% acetic acid was prepared (10), along with a neutralizing solution of two parts 10 X concentrated Medium 199 (Grand Island Biological Co., Grand Island, NY) and 1 part 0.34 N NaOH. Stock collagen solution was mixed with neutralizing solution in a ratio of 4:1, then dispensed into Falcon multiwell culture plates (1.5 cm d well).

Cells to be injected into gels were suspended at a concentration of 1.7 x 10^7 cells/ml in neutralizing collagen solution at 4°C. Three microliters of the suspension (5 x 10^4 cells) was injected, with a mouth-operated Lang-Levy pipette (VWR Scientific, Inc., San Francisco, CA), into individual preformed 1.0 ml gels. The injected gels were covered with 0.4 ml Medium 199 containing gentamicin sulfate (Sigma) and supplemented with insulin 5 μg/ml (26.4 IU/mg; Sigma) and hydrocortisone 1 μg/ml (Sigma), with or without added prolactin 5 μg/ml (ovine, NIH-P.S.11). Cultures were incubated at 37°C in 5% CO2 in air; the medium was changed on alternate days.

One-half milliliter gels served as substrates for monolayer cultures. Some gels were fixed by flooding with 2% glutaraldehyde in HBSS for 3 h, then detached from the well, rinsed in HBSS, and washed for 5 d in repeated changes of lysine-supplemented (1 mg/ml) Medium 199. Cells were seeded onto either attached, unfixed or detached, fixed gels at 5 x 10^5 cells/cm^2 in Medium 199 containing 10% bovine serum plus insulin and hydrocortisone, and incubated as above. After 48 h, half the attached gels were released by loosening the gel periphery with the flamed tip of a Pasteur pipette and swirling. Thus three gel groups were created—attached unfixed, floating unfixed, and floating fixed. In each group, half of the cultures were switched to medium with added prolactin.

**Preparation of fat pads and culture procedures.** Parenchyma-free (15) inguinal mammary fat pads were obtained from 3 to 4-wk or 6 to 8-month BALB/c female mice. One group of fat pads was fixed for 2 h in 2% glutaraldehyde in HBSS, extracted for 2 to 3 d with 70% ethanol, washed for 4 to 5 d in lysine-supplemented Medium 199, then stored at 4°C in Medium 199 containing gentamicin sulfate. A second group of fat pads was frozen solid in dry ice, then thawed in HBSS at room temperature. The freeze-thaw procedure was repeated four times, then the fat pads were washed for 4 to 5 d in Medium 199 containing 2.5 mg/ml bovine serum albumin (BSA). A third group of fat pads, removed from the hosts just before an experiment, was used alive.