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THE EFFECT OF MOUSE MAMMARY TUMOR VIRUS RECEPTOR ACTIVATION ON MAMMARY EPITHELIAL CELL SENSITIVITY TOWARD PROLACTIN

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SUMMARY: Mammary epithelial cells infected with the mouse mammary tumor virus (MMTV) require less than one-half the concentration of prolactin to elicit α-lactalbumin production than uninfected tissue (EC_{50} = 89 ± 10 ng/ml vs. 206 ng/ml, respectively). Furthermore, stimulating antibodies to the cellular receptor for MMTV halved the prolactin requirement of MMTV- tissue, while MMTV antibodies that sequestered secreted MMTV increased the prolactin requirement in MMTV+ tissue. These data suggest that the effect of MMTV on mammary epithelial sensitivity toward prolactin is being mediated by its interaction with a cell surface receptor.

Previous reports have suggested that the mammary epithelium from MMTV infected mice have altered sensitivity to sex steroids (1,2) and prolactin (3-5). In the pituitary gland, greater sensitivity toward prolactin leads to enhanced negative feedback and lower prolactin serum levels (3). In the mammary gland, increased sensitivity results in greater morphological differentiation (4) and DNA synthesis (5).

The mechanism for this difference is unknown. MMTV+ mice do have higher levels of an estradiol metabolizing enzyme, which may explain the altered sex steroid sensitivity (6). Alternatively, the MMTV may generate a gene product, directly or indirectly, that affects hormone responsiveness. The MMTV has a powerful promoter that can drive adjacent genes (7,8); but since viral insertion is random, this mechanism would be unlikely to affect the same or similar genes throughout the entire epithelium. More directly, the MMTV may possess a gene that regulates prolactin sensitivity. Originally, MMTV was deemed to be a simple retrovirus having only the standard reverse transcriptase, coat protein, and envelope genes (9); however, at least three other

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genes have been discovered: a protease (10), a superantigen (11), and a dUTPase (12). A gene encoding a transcription factor has also been proposed but not yet confirmed (13). This latter putative gene product might affect endocrine sensitivity by regulating hormone receptor levels or some aspect of the transduction machinery.

Another potential mechanism is the activation of a cell surface receptor. The MMTV infects mammary epithelium via a partially characterized plasma membrane protein (14) that appears to be coupled to certain biological activities (15). Stimulating antibodies to this receptor have been generated and can activate this protein without introducing foreign genes into the epithelial cell; as such, they are excellent tools by which a receptor mechanism could be distinguished from a genomic one.

MATERIALS AND METHODS

Ovine prolactin (oPRL-17) was kindly provided by the Hormone Distribution Program (NIADKK), and crystalline porcine insulin (lot 615-08E-220) was a gift from Eli Lilly Co. Cortisol, triiodothyronine (T_3), Hepes, UDP-galactose, galactosyltransferase, and α-lactalbumin were purchased from Sigma Chemical Co. Medium 199 with Hanks' salts and normal rabbit serum were obtained from Grand Island Biological Company and UDP-[6-^{3}H]galactose (18.9 Ci/mmol) was from Amersham Corp.

Virgin mice (C3H/HeN) were purchased from the National Cancer Institute. Newborn female mice from MMTV- mothers were randomly kept with their MMTV- mothers or cross-fostered by MMTV+ lactating dams. When the mice were 3 months old, they were killed by cervical dislocation and explants were sterilely prepared from the fourth pair of mammary glands as previously described (16). The explants were cultured on siliconized lens paper in medium 199 containing 20 mM Hepes (pH 7.6), insulin (1 μg/ml), cortisol (1 μg/ml), T_3 (65 pg/ml), and varying concentrations of prolactin (25-5000 ng/ml). Some culture media also contained antiserum to either the envelope protein of MMTV or the MMTV binding protein; the preparation and validation of these antisera have been reported elsewhere (14,17). The concentration of antiserum was 10 μl/3 ml and control dishes had normal rabbit serum. The tissue was incubated under air at 37° and the media was changed daily.

α-Lactalbumin was assayed in mammary explants by a modification (18) of the method of Fitzgerald et al. (19) using bovine α-lactalbumin as standard.

RESULTS

Prolactin dose-response curves were sigmoidal with a central linear segment (Fig. 1); this segment was subjected to regression analysis in order to estimate the EC_{50} for α-lactalbumin induc-

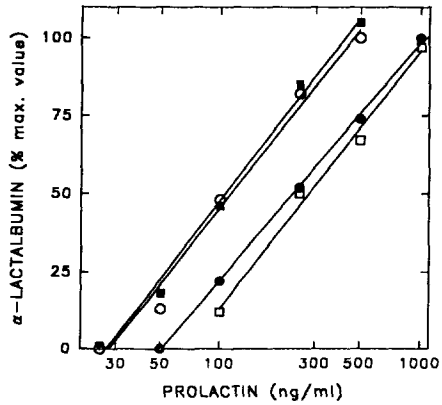

Figure 1. Prolactin dose-response curves for α-lactalbumin induction in explants from MMTV+ (square) and MMTV- mice (circle). The explants were cultured with insulin, cortisol, T_3, prolactin, and either normal rabbit serum (solid symbols) or antiserum to either the MMTV receptor (open circle) or the MMTV envelope protein (open square).

tion. The prolactin EC_{50} for explants from MMTV+ mice is less than half that for tissue from MMTV- animals (Table 1). If this effect were due to secreted MMTV acting at the cell surface, then antibodies to the MMTV envelope protein would block viral binding to its receptor and raise the EC_{50}. This is exactly the result

TABLE 1

The Effect of MMTV Infection and Antisera on Epithelial Cell Sensitivity Toward Prolactin

Mice	Antiserum	EC_{50} for α-lactalbumin (ng/mg wet tissue)
MMTV-	0	206 ± 16
	+	91 ± 12
MMTV+	0	89 ± 10
	+	264 ± 19

Female C3H/HeN mice were nursed by either MMTV+ or MMTV- mothers. At maturity, mammary explants were cultured in insulin, cortisol, T_3 and varying concentrations of prolactin. The antiserum was either to the MMTV receptor (MMTV- mice) or the MMTV envelope glycoprotein (MMTV+ mice). After 3 d, α-lactalbumin was determined and the prolactin concentration required for 50% maximal stimulation (EC_{50}) was calculated. Each value is the mean ± S.E. for four separate experiments.

obtained; indeed, the values for the MMTV- control are very similar to the MMTV+ explants cultured with antiserum.

Conversely, MMTV binding to the cell surface of MMTV- tissue should increase the prolactin sensitivity of these explants. Unfortunately, because it takes 3-4 d to get maximal induction of α-lactalbumin, there is a chance that the MMTV could successfully infect the epithelial cells. The resulting gene products would confound the interpretation of the data. Therefore, stimulating antibodies to the MMTV receptor were used; this antiserum can activate the receptor without introducing foreign genes into the cell (15). These antibodies lower the prolactin EC_{50} in MMTV- explants to values seen in MMTV+ tissue (Table 1).

DISCUSSION

Viruses propagate themselves by hijacking the cellular processes of their hosts for their own benefit. Although this control is most often achieved internally via gene regulation, there is increasing evidence that viruses can exert external control as well. Specifically, viruses can bind to a variety of cell surface proteins (20,21). Although this binding provides a mechanism for the virus to gain entry into its target tissue, some of these viral receptors are coupled to transduction systems that are activated by viral binding (see Discussion in Ref. 15).

This also appears to be true for the MMTV: e.g., a previous report has shown that the exposure of leukemia cells to extracellular MMTV increased viral production (22); the brevity of the exposure suggested a receptor-mediated event. Furthermore, this laboratory has demonstrated that stimulating antibodies to the MMTV receptor can enhance amino acid uptake and total RNA synthesis (15), as well as increase the prolactin sensitivity of the mammary epithelium (Table 1). These activities would favor mammary gland milk production and the secretion of the MMTV, thereby favoring the propagation of the MMTV to the next generation. Unlike the intact virus, these antibodies do not introduce additional genes into the epithelium; therefore, the elicited activities appear to be a direct result of the activation of plasma membrane binding proteins.

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