Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 579–582

Laminin and collagen IV enhanced casein synthesis in bovine mammary epithelial cells

T. Yano[1,3], H. Aso[2], K. Sakamoto[1], Y. Kobayashi[1], A. Hagino[1], K. Katoh[1] and Y. Obara[1]

[1]Department of Animal Physiology, [2]Cellular Biology Laboratory, Division of Life Science, Graduate School of Agricultural Science, Tohoku University Amamiyamachi, Aoba-ku, Sendai 981-8555 Japan

ABSTRACT

In the bovine mammary gland, extracellular matrix proteins such as collagen I and IV and laminin coexist around the alveoli during lactation. In contrast, connexin, a gap junction protein, was scarce in lactating mammary epithelial cells. In addition, collagen IV and laminin were shown to be involved in alpha-casein synthesis and secretion in the bovine mammary epithelial cells. The connexin proteins, which were detected during the non-pregnant and dry periods, were scarce at peak lactation. It was shown that extracellular matrix proteins are important for the stimulation of casein synthesis, but this was accompanied by a suppression of connexin expression.

KEY WORDS: bovine, extracellular matrix, collagen IV, laminin, connexin, casein synthesis

INTRODUCTION

The extracellular matrix (ECM), which is essential for mammary gland function, dramatically changes during lactational stages, and its expression is under the influence of ovarian hormones (Berry et al., 2003). Collagen I (CL I) is known to stimulate alpha S1-casein synthesis in primary bovine mammary epithelial cells (Delabarre et al., 1997). Furthermore, laminin (LN) is known to influence beta-casein synthesis in a mouse mammary epithelial cell line (Streuli et al., 1995). ECM influences not only casein synthesis but also expression of connexin (Cx), which is a component of gap junction proteins. In rodent mammary glands, the gene and protein expression of Cx 43, 32 and 26 were greater during lactation, but the expression of Cx 32 and 26 were detected in the non-pregnant human

[3] Corresponding author: e-mail: tomoky@bios.tohoku.ac.jp

mammary gland (Pozzi et al., 1995). Thus, although there might be differences in the use of Cx among animal species, the expression is probably closely related to lactogenesis. In the present study, we studied the expression of the proteins for ECM and gap junctions among different developmental stages of the bovine mammary gland, and the effects of ECM on casein synthesis and secretion in a bovine mammary epithelial cell line (bMEC), which was established in our laboratory.

MATERIAL AND METHODS

Immunohistochemistry was carried out with bovine mammary tissues taken from non-pregnant, peak lactation, late lactation, and dry dairy cows for CL I, CL IV, LN, Cx 43, Cx 32 and Cx 26. bMEC were cultured in Transwell insert wells coated by CL I, CL IV and LN as described date sheet. Culture medium was made with or without lactogenic hormones (DIP: dexamethasone 10µl/ml, insulin 10 µl/ml and prolactin 10 µl/ml). Conditioned medium collected on day 3, 6, and 9 was used for the determination of alpha-casein ccentratins by ELISA. Cell lysate of bMEC was used for Western blot analysis of Cx 43 (Zymed Lad. Inc.) and Cx26 (Chemincon Int.).

RESULTS

Expression of CL I, CL IV and LN was identified around the alveoli at peak lactation. This finding suggests that these ECM proteins are involved in milk protein synthesis such as casein. Based on this finding, we investigated the influence of ECM proteins on alpha-casein synthesis in bMEC. Alpha-casein secretion was much increased when cultured in CL IV- or LN-coated wells, even in the absence of DIP. Alpha-casein levels were stimulated to a greater extent still by CL IV- or LN-coating in the presence of DIP.

Although the expression of Cx43 and 26 was detected during the non-pregnant and dry periods, it was not detected during peak or late lactation. Cx32 was detected in the mesencyma but not in the parenchyma. These findings were different from the expression of ECM proteins. Therefore, in bMEC cultured on these ECM proteins, although the Cx26 expression was increased in CL I-coated dishes, despite no change in alpha-casein synthesis, the decreased expression of Cx26 in CL IV- and LN-coated dishes was related to the increase in alpha-casein synthesis.

DISCUSSION

The present study clearly showed that CL IV and LN, which are the major components of the basement membrane of the mammary gland, strongly

stimulated casein synthesis in bMEC. However, this result does not concur with a previous finding in primary bMEC (Delabarre et al., 1997), but was consistent with a result in a mouse mammary epithelial cell line (Streuli et al., 1995). In the bovine mammary gland, the expression of Cx was decreased during lactation, which was influenced by ECM proteins. In a mouse mammary epithelial cell line, as the casein protein synthesis was increased, the expression of Cx 43 was increased (El-Sabban et al., 2003). In addition, the expression of Cx was increased during lactation in the rodent mammary gland, and was detected in the human non-pregnant mammary gland (Pozzi et al., 1995). Eventually, the expression of Cx influenced by ECM proteins may vary among animal species and lactation stages.

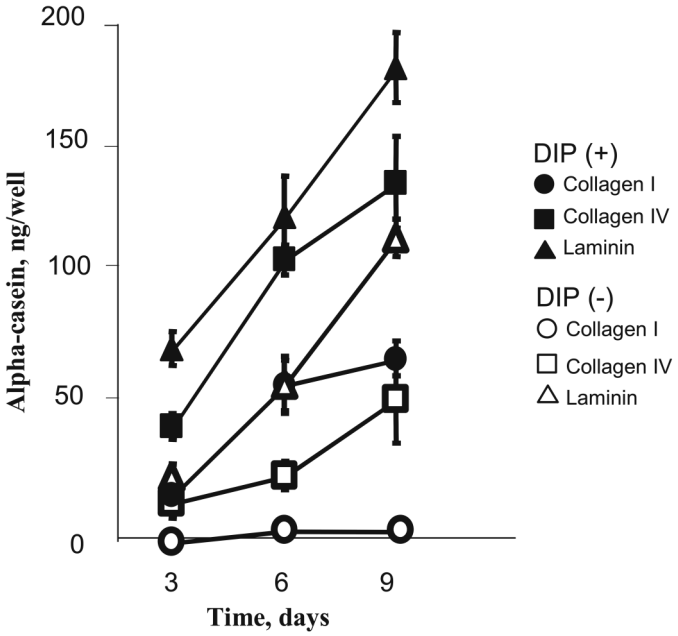

Figure 1. Effect of ECM and DIP on alpha-casein secretion from cultured bMEC

CONCLUSIONS

It was clearly shown that variations in the expression of ECM proteins and gap junction proteins are important for mammary differentiation. The results presented here indicate that CL IV and LN stimulated alpha-casein synthesis and secretion, but decreased the expression of Cx 26. These results support the implication that the expression of ECM proteins is raised during lactation, which is accompanied with depressed Cx.

REFERENCES

Berry S.D., Howard R.D., Akers R.M., 2003. Mammary localization and abundance of laminin, fibronectin, and collagen IV proteins in prepubertal heifers. J. Dairy Sci. 86, 2864-2874

Delabarre S., Claudon C., Laurent F., 1997. Influence of several extracellular matrix components in primary cultures of bovine mammary epithelial cells. Tissue Cell 29, 99-106

El-Sabban M.E., Sfeir A.J., Daher M.H., Kalaany N.Y., Bassam R.A., Talhouk R.S., 2003 ECM-induced gap junctional communication enhances mammary epithelial cell differentiation. J. Cell Sci. 116, 3531-3541

Pozzi A., Risek B., Kiang D.T., Gilula N.B., Kumar N.M., 1995. Analysis of multiple gap junction gene products in the rodent and human mammary gland. Exp. Cell Res. 220, 212-219

Streuli C.H., Schmidhauser C., Bailey N., Yurchenco P., Skubitz A.P., Roskelley C., Bissell M.J., 1995. Laminin mediates tissue-specific gene expression in mammary epithelia. J. Cell Biol. 129, 591-603