Distribution of Cytokeratin Polypeptides Detected by Monoclonal Antibodies K8.13 and K8.12 in the Fetal Bovine Ruminal Epithelium

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ABSTRACT. Temporal and spatial distributions of cytokeratin (CK) polypeptides were detected by monoclonal antibodies (mAbs) K8.13 and K8.12 during the development of the bovine ruminal epithelium. By the Western blotting analysis after the sodium dodecyl sulfate-polyacrilamide gel electrophoresis, mAb K8.13 confirmed 60.8 and 63.0 kD CK polypeptides in the fetal ruminal epithelial extract, and mAb K8.12 also 48.0 and 54.0 kD CK polypeptides. Immunohistochemical reactivities against both mAbs were detected only in the epithelial cells throughout the fetal periods. Distributions of CK polypeptides detected only by mAb K8.13 were observed on the basal side of the epitherial layer, but not by mAb K8.12 in the 7 cm fetus in crown-rump length. MAb K8.13 reacted also intensely with columnar-shaped cells in the basal layer in the fetuses of the later developmental periods. These results suggest that CK polypeptides detected by mAb K8.13 might be involved in the differentation and/or the maintenance of the basal layer in the ruminal epithelial development.—KEY WORDS: bovine, cytokeratin, development, epithelium, rumen.

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We have been interested in the mechanism of the morphogenetical regulations under the epithelial-mesenchymal interaction, and have studied the morphogenesis of the bovine ruminal papillae and the palatine ridge [1, 2, 16–18]. The epithelial-mesenchymal interaction is known to conduct several morphogenetic processes such as the digestive tract [21] and the chick feather germ [11]. The finger- and/or leaf-like ruminal papillae have regulary aligned on the ruminal inner surface. We have studied temporal and spatial distributions of extracellular matrix such as fibronectin, laminin, chondroitin sulfate proteoglycans and some collagens as the marker of the mesenchymal differentiation, and those of carbonic anhydrase isozyme III as the marker of the epithelial one in the ruminal papillae (RP) [1, 16, 17] and palatine ridge (PR) [2, 17] of bovine fetuses. We have revealed that distributions of fibronectin, some condroitine sulfate proteoglicans and carbonic anhydrase isozyme III might be correlated with the morphogenetic processes of the RP and the PR [1 ,2, 17, 18]. Ruminal surface is covered with a non-keratinized stratified squamous epithelium, and cytokeratin (CK) polypeptides are known to be expressed in response to the epithelial differentiation during the keratinizing process [6]. We planned to examine temporal and spatial distributions of CK polypeptides to obtain some differentiational informations in the developing RP. CK polipeptides is known to be an intermediate filament in the epithelium. Bovine CK family consists of about 20 polypeptides [7, 9, 15]. Skin-type (56.5/62.0–65.0 kD) [13], esophagus-type (43.0/58.0 kD) [14], hyperproliferated epithelial-type (46.0/57.0 kD) [20] and simple layered epithelial-type CK polypeptides [7] have been characterized as markers for various epithelial differentiations. In this study, we examined the relationship between the distributions of CK polypeptides and the histogenesis of the ruminal epithelium using two mAbs ; i.e., MAb K8.13 raised against the stratified epithelial type CK polypetides [10, 13, 14], and mAb K8.12 against the non-stratified hyperproliferative epithelial type CK polypeptides [10, 20]. These mAbs recognize the broad-range of CK polypeptides in species including man, cow, chick and amphibia, but show only limited reactivity with only a few rodent CK polypeptides [10].

MATERIAL AND METHODS

Sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) and Werstern blotting: Fourteen bovine fetuses from 7 to 90 cm in crown-rump length (CRL) were obtained from a local slaughterhouse, Saitama Prefecture, Japan (Table 1). The ruminal epithelium was homogenized in 50 mM Tris-HCl, pH 7.4, with 1 M KCl, 1 mM ethylenediaminetetraacetic acid disodium salt, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreit and 1% Triton X-100 (all reagents were purchased from Sigma Chemical Co., Ltd., U.S.A.), stirred at 4°C overnight, and then centrifugated at 18,000 g for 30 min at 4°C. The precipitate was resolved in the Laemmli's buffer [12]

Table 1. Bovine rumen used in the present study

Sample No.	CRL (cm)	Sample No.	CRL (cm)
1	7	9	35
2	9	10	47
3	15	11	55
4	18	12	69
5	21	13	74
6	24	14	90
7	28		
8	32		

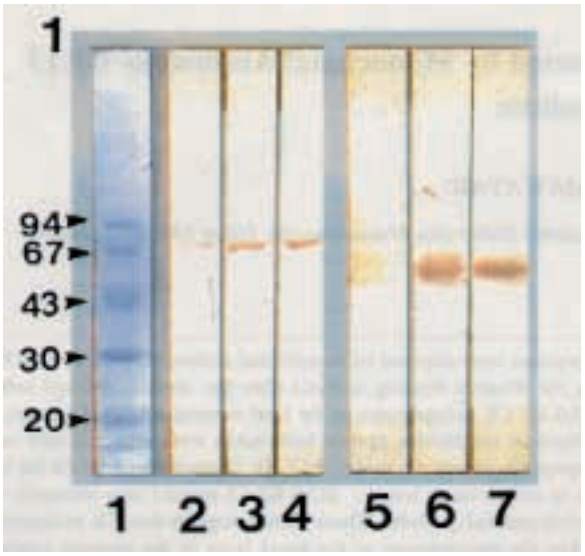


Fig. 1. CK polypeptides from the fetal rumen (CRL 30, 57, 78 cm) by the Western blotting (10% SDS-PAGE). Lane 1 shows marker proteins stained by a coomassie blue. Lane 2 and 5 are the immunoreactions in the fetus of CRL 30 cm, lane 3 and 6 are in the fetus of CRL 57 cm and lane 4 and 7 are in the fetus of CRL 78 cm. MAb K8.13 reacts with 60.8 and 63 kD CK polypeptides (Lane 3, 4, 5). MAb K8.12 reacts with 48 and 54 kD CK polypeptides (Lane 5, 6, 7). Immunoreactions to both mAbs are gradually increased from the fetuses at CRL 30 to 78 cm.

containing 8 M urea, and then reduced in boiling water for 10 min. CK samples were run on a 10% SDS-PAGE, and transferred onto the Immobilon membrane (Millipore, U.S.A.) by the Western blotting. The transferred membranes were incubated with each mAb against CK polypeptides (K8.13 or K8.12; BioMakor, Israel), and immunoreaction was detected by the indirect immunodetectional method (anti-mouse IgG conjugated with horseradish peroxidase; Seikagaku Kogyo, Japan). After rinsing, peroxidase activity was colored by 3,3'-diamino-benzidine.

Immunohistochemistry: Samples from the atrium ruminis were fixed in 4% paraformaldehyde in 100 mM phosphate buffered saline (PBS), pH7.4, at 4°C for 4 hr. After rinsing in 10 mM PBS, samples were treated with a graded series of sucrose in 10 mM PBS (10–20% sucrose), frozen in dry ice-aceton and sectioned at 5 μm with a cryotome. Each section was rinsed in 10 mM PBS, blocked non-specific immunoreaction in 5% bovine serum albumin (Sigma Chemical Co., Ltd., U.S.A.), and incubated with mAbs K8.13 or K8.12 for 90 min at room temperature. Primary antibodies were detected by the avidin-biotin-complex method [5], and observed with a light microscope.

RESULTS

Western Blotting: MAb K8.13 gave two bands at 60.8 and 63.0 kD, and mAb K8.12 at 48.0 and 54.0 kD in the epithelial extract of the fetal rumen on the transferred membranes. The immunoreactivities against both mAbs were gradually increased during the fetal developmental stages (Fig. 1).

Immunohistochemistry

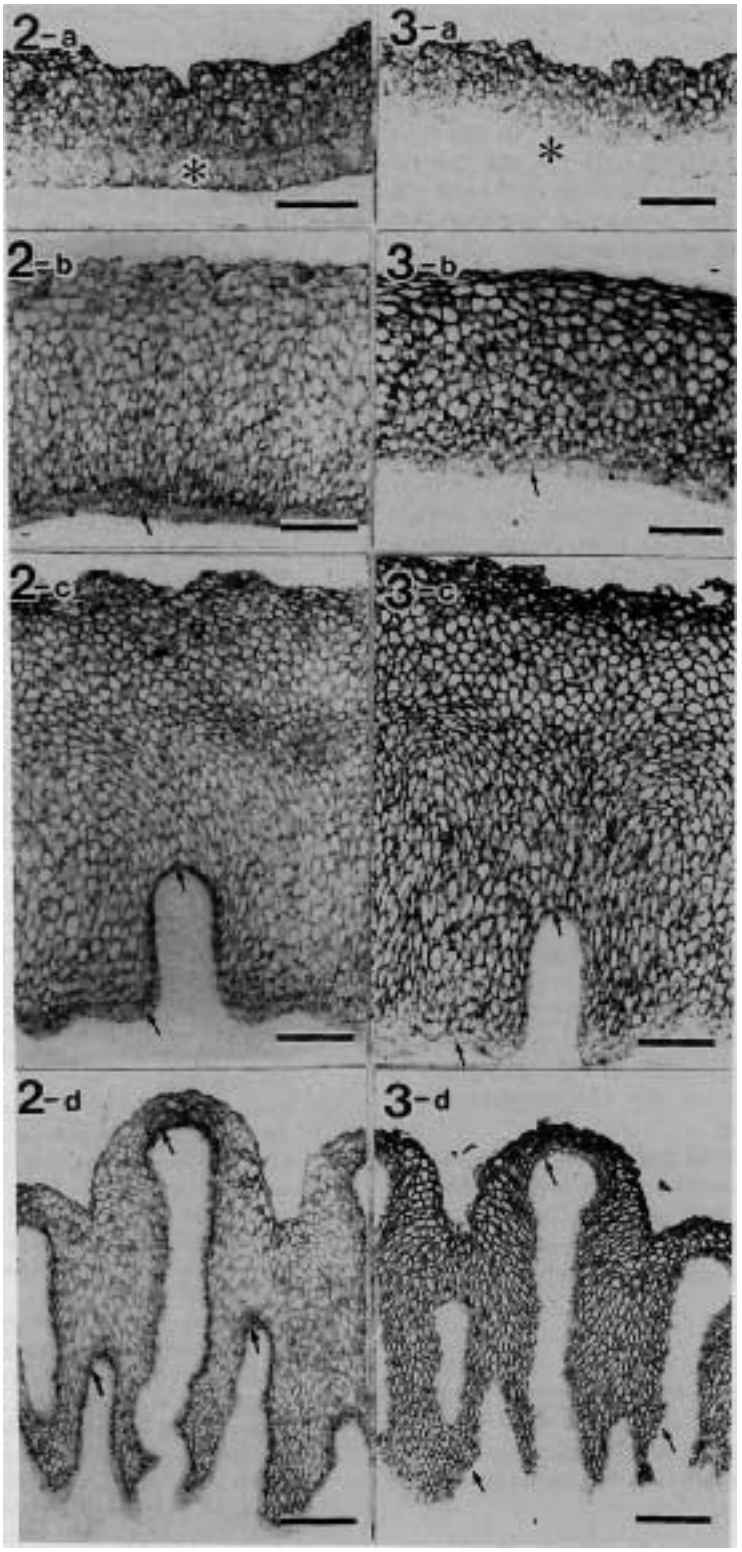
Stage 1: the periods before detection of the epithelial basal layer (fetuses less than 10 cm CRL): The epithelial basal layer was not completely formed and the epithelial cells were varied in size and shape. The immunoreactivities of mAbs K8.13 and K8.12 were stronger in the upper regions of the epithelial layer than in the basal one (Figs. 2a, 3a). The immunoreaction of only mAb K8.13 was detected on the basal side of the undifferentiated epithelial cells (Fig. 3a, asterisk).

Stage 2; the periods after detection of the basal layer (fetuses more than 15 cm CRL): The epithelial basal layer was observed just above the interface between the epithelium and the mesenchyme (Figs 2b, 3b). In the fetuses of 25–28 cm CRL, small papillae of the lamina propria protruded into the epithelial layer (data not shown). The epithelial cells in the basal layer were typically columnar in shape. The spinndle-shaped cells were observed in the suprabasal layer (Figs 2c, 3c). The surface epithelial cells at the top of the RP were flat in shape in the fetuses more than 45 cm CRL (Figs. 2c, 2d, 3c, 3d). MAb K8.13 reacted more intensely with the basal layer cells than the suprabasal cells (Fig. 2b, 2c, 2d). The immunoreactivities by mAbs K8.12 were intense in the suprabasal epithelial layer in the fetuses more than 45 cm CRL (Figs. 3c, 3d).

DISCUSSION

Histological studies have indicated that keratohyalin granules were detected in the superficial cells of the developing ruminal epithelium in the fetuses more than 45 cm CRL, suggesting that the ruminal epithelium was developed similarly to the stratified epithelium [3, 4]. However, temporal and spatial distributions of CK polypeptides in the developing rumen are not known yet. We examined the immunohistochemical distributions of CK polypeptides to obtain some informations about the epithelial cellular differentiation in the fetal bovine rumen. Prior to the immunohistochemical stainings, we examined the molecular weight of CK polypeptides detected by mAbs K8.13 and K8.12 in the ruminal extract of the bovine fetuses by the Western blotting, because both mAbs reacted with many types of CK polypeptides. MAb K8.13 reacted with

Figs. 2 and 3. The immunostaining by mAbs K8.13 (2-a, -b, -c, -d) and K8.12 (3-a, -b, -c, -d) in the fetal ruminal epithelium. (a) CRL 7 cm fetus; the epithelial basal layer is not formed. Immunoreactivities of both mAbs are stronger in the upper epithelial region than the lower one. Only mAb K8.13 reacts on the lower side of the undifferentiated epithelial layer. (b) CRL 30 cm fetus; the basal epithelial layer is formed. Immunoreactivities of both mAbs are intensely observed in the upper epithelial layer. Immunoreactivities of only mAb K8.13 is detected in the cytoplasm of the basal cells. (c) CRL 54 cm fetus; small ruminal papillae are detected. Immunoreactivities of mAb K8.13 are stronger in the cytoplasm of the basal cells than the sprabasal one,



and those of mAb K8.12 are more intense in the suprabasal layer than the basal one. (d) CRL 78 cm fetus; well-developed ruminal papillae are observed on the luminal surface. Immunoreactivities of mAb K8.13 are more intense in the cytoplasm of the basal cells than the suprabasal one, and those of mAb K8.12 are intense in the suprabasal layer. bar: 100 μm in a, b and c, and 50 μm in d. Arrows indicat the basal cells.

60.8 and 63.0 kD CK polypeptides in the bovine ruminal extract, and mAb K8.12 also with 48.0 and 54.0 kD. In humen, mAb K8.13 reacts with Nos. 1 (62.0–65.0 kD), 5 (58.0 kD), 6 (56.0 kD), 7 (54.0 kD), 8 (52.0 kD), 10 (56.5 kD), 11 (56.0 kD) and 18 (45.0 kD) [13, 14], and mAb K8.12 with Nos. 13 (51.0 kD), 15 (50.0 kD) and 16 (48.0 kD) CK polypeptides [8, 20]. Although all bovine CK polypeptides do not always correspond to human CK polypeptides, the molecular weights are closely resembled with each other [13, 15]. As compared to these CK polypeptides, mAb K8.13 might recognize No.1 CK (62.0–67.0 kD) in bovine CK, and mAb K8.12 No.15 (50.0 kD) and/or 16 (46.0 kD) CK polypeptides. Bovine and human No. 1 CK is known to be the skin-type CK and regarded as a marker for the keratinized stratified epithelium; *e.g.*, the epidermis and the hoof [14]. In the present study, it was noted that CK polypeptides detected by mAb K8.13 strongly reacted with the epithelial cell in the basal layer. Before the formation of the epithelial basal layer, immunoreactions with mAb K8.13 was stronger on the basal side of the undifferentiated epithelial cells than the suprabasal side. After formation of the epithelial basal layer, CK polypeptides detected by mAb K8.13 were densely distributed in the epithelial basal cells. On the other hand, mAb K8.12 was weakly reacted with the epithelial basal cells. Expressions of CK polypeptides reacted with both mAbs by the Western blotting were correlated with the immunohistological reactions. These results revealed that the cytoskeletal elements were different in the fetal ruminal epithelial cells between the basal and the suprabasal layers. Such differences of the cytoskeletal elements might reflect the morphological and/or the functional differences; *e.g.*, the cell-shape or the cell cycle speed between the basal and the suprabasal layers in the developing rumen. These results are also supported by the abnormal CK expressions in the hyperploriferative disorder of the human epidermis [20], and the developmental expressions of CK polypeptides in the chick digestive tract; *e.g.*, the differentiation of the gastric glandular cells [19].

In conclusion, we revealed that some CK polypeptides detected by mAb K8.13 were intensely expressed in the basal cells in the fetal bovine rumen, suggesting the morphological and/or the functional differences of the cells between the basal and the suprabasal layers in the bovine fetal rumen.

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