Detection of betaglycan in porcine and human milk

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Betaglycan is a transmembrane proteoglycan with a high binding affinity to transforming growth factor beta (TGF-,). It may play a specific role in TGF-, signal transduction by presenting TGF-, to the receptors (1). Soluble form of betaglycan has been detected in various biological fluids, including cell culture medium, cellular matrix extract (2) and rat milk (3). It has been suggested that soluble betaglycan may act as a dual modulator regulating the binding of TGF-, to the surface receptors (4) and neutralizing excessive ligands (5). In the present study, betaglycan was detected in porcine and human milk by Western blot analysis.

Porcine milk samples were collected from four Large White sows at day one, day three, day seven and day fourteen of lactation. Human milk was collected from a lactating mother during the second month of lactation. Fat in the samples was removed by centrifugation and protein concentration was determined by Lowry's method. Characterization of betaglycan in porcine and human milk was performed by western blot analysis under non-reducing and reducing conditions. Polyclonal antibodies against betablycan (Santa Cruz Biotechnology) was used in the analysis.

Immunoreactive betaglycan was detected in all milk samples. In porcine milk the concentration of betaglycan was highest on the first day of lactation and the concentration declined gradually with the progress of lactation (Figure).

Figure 1. Western blot analysis under non-reducing condition. Lane 1, isolated human betaglycan. Lanes 2–4, porcine milk at day 1, 3, 7, 14 respectively. Lane 5, human milk.

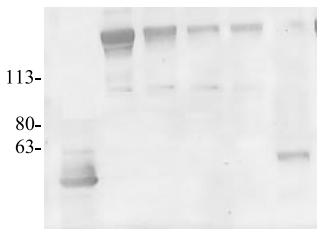

Figure 2. Western blot analysis under reducing condition. Lane 1 isolated human betaglycan, Lanes 2–4, porcine milk at day 1, 3, 7, 14 respectively. Lane 5, human milk.

The estimated molecular size of immunoreactive betaglycan in porcine milk was about 180 kDa. Under reducing condition, the molecular size of immunoreactive betaglycan reduced to below 60 kDa, indicating the existence of disulphide bonds in the original compound. Incubation of porcine milk with deglycosylation enzymes, chondritinase and heparitinase reduced the molecular size from 180 kDa to about 110 kDa, confirming the existence of chondroitin sulphate and heparan sulphate glycosaminoglycan chains (3). In contrast, the immunoreactive betaglycan detected in human milk had a molecular size of around 75 kDa and it did not change under reducing condition. The difference between human and porcine milk in the molecular characteristics of immunoreactive betaglycan may be due to a species difference or to post-excretory degradation of the compound.

This study represents the first report of betaglycan in porcine and human milk. The physiological significances of milk-borne betaglycan remain to be elaborated.

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