Human milk fibronectin: identification of fibronectin fragments by transfer of milk proteins from polyarcrylamide gels to nitrocellulose sheets

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1. INTRODUCTION

The term 'fibronectin' describes a family of structural and immunologically related high-M_r glycoproteins that are present in vertebrates in both the soluble and insoluble form. The soluble form has been observed in various biological fluids including plasma, cerebrospinal, amniotic and sinovial fluids, urine and seminal plasma [1–6]. The insoluble form is present in vertebrate tissue, intracellularly, and as an extracellular matrix component [7–12].

Although little is known about the function of fibronectin in vivo, it has been shown to display many interesting features in vitro ([13–20] and references therein). The amount of fibronectin present on the surface of transformed or neoplastic cells is generally greatly reduced with respect to their normal counterparts. Fibronectin promotes cell aggregation and cell substratum adhesivness, partially restores transformed fibroblasts to a more normal phenotype, promotes locomotion of certain types of cells and facilitates reticuloendothelial system clearance of particles.

Here, we report that human milk contains fibronectin and other components of lower M_r which are recognized by anti-fibronectin antibodies. These lower M_r components are most probably fibronectin fragments.

2. MATERIALS AND METHODS

Milk was obtained from 12 healthy women between day 2 and 15 following delivery. Samples of 2 ml each were centrifuged at 10 000 × g for 30 min and the cream removed (fat-free milk). Aliquots were then respun at 90 000 × g for 30 min to remove casein (lactoserum). All samples were analyzed within 24 h after drawing.

Rabbit monospecific and mouse monoclonal anti-human plasma fibronectin antibodies were prepared as in [21,22].

Immunodiffusion analysis was done in 1% agarose using the double diffusion method [23]. To estimate the amount of fibronectin in fat-free milk and lactoserum we have used the solid-phase double-antibody radioimmunoassay in [24]. Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was done as in [25]. The transfer of protein from SDS–PAGE to nitrocellulose sheets and the immunoenzymatic detection of fibronectin was performed as in [26]. Heparin–toluidine blue staining was used to locate transferred proteins on the nitrocellulose sheets [27]. This reversible staining procedure gives a pattern similar to that obtained with Coomassie brilliant blue R250 and the same nitrocellulose sheet, after destaining, can be used for immunoenzymatic studies (Towbin, personal communication).

3. RESULTS

Fat-free milk samples reacted with monospecific rabbit antibodies to human plasma fibronectin in Ouchterlony immunodiffusion analysis showing immunological identity with human plasma fibronectin (fig.1).

Milk and plasma total protein, resolved by SDS–PAGE, were transferred to nitrocellulose

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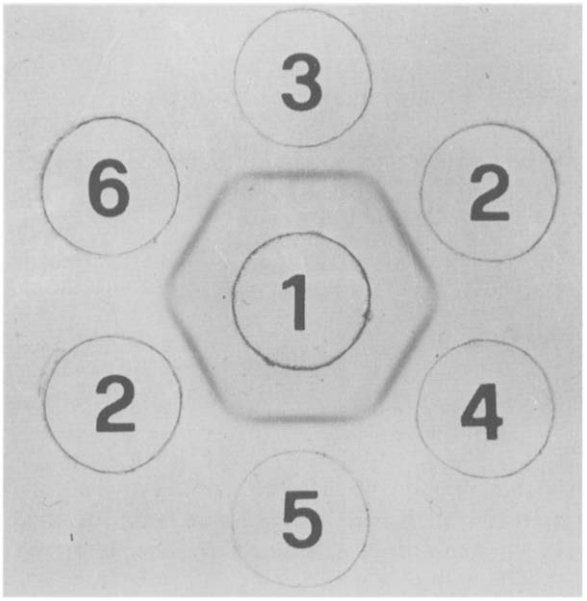

Fig.1.Immunodiffusion assay in 1% agarose: (1) Rabbit antibodies to human plasma fibronectin; (2) human serum diluted 1/50; (3,5) two different undiluted fat-free milk samples; (4,6) purified human plasma fibronectin.

Table 1

Concentration of fibronectin in milk from different subjects

Sample no.	Days after delivery	μg Fibronectin/ml milk
1	11	1.7
2	2	3.7
3	4	4.2
4	7	6.4
5	8	1.8
6	3	6.8
7	9	6.2
8	4	6.8
9	3	1.5
10	3	1.8
11	7	8.1
12	6	12.2

sheets. The fibronectin bands were subsequently identified immunoenzymatically. This procedure gives information (subunit composition M_r-value, possible presence of fragments) on antigens in any biological fluid avoiding artifacts due to the purification procedure.

Fig.2A represents a nitrocellulose sheet, on which total serum and milk proteins, after separation on a 10% SDS–PAGE, were transferred and stained by the heparin–toluidine procedure. Fig.2B shows the same sheet as in fig.1A after destaining and immunoenzymatic localization of fibronectin as in section 2. Human plasma gave the two typical fibronectin polypeptide bands in the 220 000 M_r region. In the case of milk, the same two 220 000 M_r fibronectin bands were always accompanied by a certain

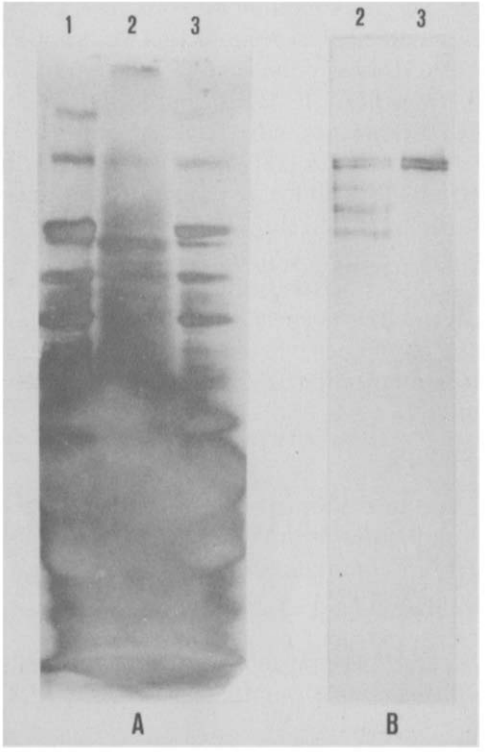

Fig.2. Electrophoretic transfer and localization on nitrocellulose sheets of milk and plasma fibronectin. (A) Nitrocellulose sheets on which human serum proteins (lanes 1,3) and fat-free milk proteins (lane 2) were transferred after separation on a 6% SDS–polyacrylamide gel electrophoresis. Proteins were stained with the heparin–toluidine blue procedure as in section 2. (B) The same sheet from A (lanes 2,3) after destaining and immunoenzymatic localization of fibronectin as in section 2.

number of lower M_r bands, probably representing fibronectin fragments.

The amount of fibronectin present in the milk was measured using the solid phase radioimmunoassay system section 2. Table 1 shows the fibronectin concentration in 12 different milk samples. A variation ranging from 1.7–12.2 μg/ml between the different samples was observed. No difference was observed for the fibronectin levels in fat-free milk and lactoserum where casein had been removed by ultracentrifugation.

4. DISCUSSION

Here we show that human milk contains fibronectin which is immunologically identical to plasma fibronectin. Using a radioimmunoassay system we have estimated that fibronectin concentrations in milk range from 1.2–12.7 μg/ml as compared to 400–500 μg/ml for human plasma.

In [28] fibronectin was reported in bovine colostrum but fibronectin in bovine milk was not observed. In the limited number of samples analyzed we did not observe any correlation between fibronectin concentration and time elapsed (from 1–12 days) after delivery. We are presently studying fibronectin in milk in a larger number of subjects at different times following delivery.

The origin of the fibronectin found in milk is unknown. It could originate from local synthesis by mammary epithelial cells [29,30] or macrophages known to be present in milk. Milk fibronectin could also originate from filtration of the blood.

Fractionation by SDS–PAGE of total fat-free milk followed by transfer of the protein to nitrocellulose sheets and immunoenzymatic staining demonstrated a limited number of fibronectin fragments which could not be detected in plasma. The origin of these fragments is unclear. One hypothesis is that milk fibronectin may have a different carbohydrate composition with respect to plasma fibronectin, making it more sensitive to proteolytic enzymes.

It is not prudent to speculate on the possible biological functions of either intact fibronectin or fibronectin fragments in milk. Nevertheless, due to the many biological activities observed in vitro, we can not exclude the possibility that milk fibronectin may play an important role in either the development or defense mechanisms of the new-born child.

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