

COW'S MILK PROTEINS IN HUMAN MILK

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Cow's milk proteins (CMPs) are among the best characterized food allergens. Cow's milk contains more than twenty five different proteins, but only whey proteins α -lactalbumin, β -lactoglobulin, bovine serum albumin (BSA), and lactoferrin, as well as the four caseins, have been identified as allergens. Aim of this study was to investigate by proteomics techniques cow's milk allergens in human colostrum of term and preterm newborns' mothers, not previously detected, in order to understand if such allergens could be cause of sensitization during lactation. Term colostrum samples from 62 healthy mothers and preterm colostrum samples from 11 healthy mothers were collected for this purpose. The most relevant finding was the detection of the intact bovine alpha-S1-casein in both term and preterm colostrum. Using this method, which allows direct proteins identification, β -lactoglobulin was not detected in any of colostrum samples. According to our results bovine alpha 1 casein that is considered a major cow's milk allergen is readily secreted in human milk: further investigations are needed in order to clarify if alpha-1-casein has a major role in sensitization or tolerance to cow's milk of exclusively breastfed predisposed infants.

Cow's milk contains more than twenty five different proteins, but only whey proteins α -lactalbumin, β -lactoglobulin, bovine serum albumin (BSA), and lactoferrin, as well as the four caseins, have been identified as allergens (1). In particular, the casein fraction is composed of α S1-, α S2-, β -, and κ -casein, of which α S1-casein seems to be the major allergen according to IgE and T cell recognition data (2-4).

By now the detection of the above mentioned food allergens in human milk has been achieved by sandwich Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoblotting techniques. (3). ELISA and Immunoblotting techniques have the disadvantage of possible cross reactions with other proteins that could be present in the sample and don't take into account the possible chemical modification or proteolytic digestion of the proteins (5). Aim of this study was to investigate cow's milk allergens in human colostrum of term and preterm newborns' mothers by proteomics techniques in order to understand if cow's milk allergens could be a cause of sensitization established through lactation.

MATERIALS AND METHODS

Term colostrum samples were collected from 62 healthy mothers delivering at term (36 completed week of gestation).

Preterm colostrum samples were collected from eleven healthy mothers who delivered prematurely (between 25 and 30 weeks of gestational age).

All samples were collected from mothers following a non restricted diet including cow's milk and derivatives, just after breastfeeding their own babies.

Samples were gathered up until the 4th day after delivery and frozen and stored at -80°C immediately after collection. Before analysis samples were defrosted at room temperature. Two different pools of 245 mL were created, respectively one of Colostrum of mothers delivering Term infants (CT) and another one of Colostrum of mothers delivering Preterm infants (CP). Then in each pool (CT and CP) five complete protease inhibitor cocktail tablets were added.

The sensitive detection of less represented new types of proteins contained in the whey fraction was made through the introduction of an additional prefractionation step. This step theoretically increases the concentration of the most diluted proteins and simultaneously reduces the concentration of the

Key Words: cow's milk proteins, proteomics techniques, allergens

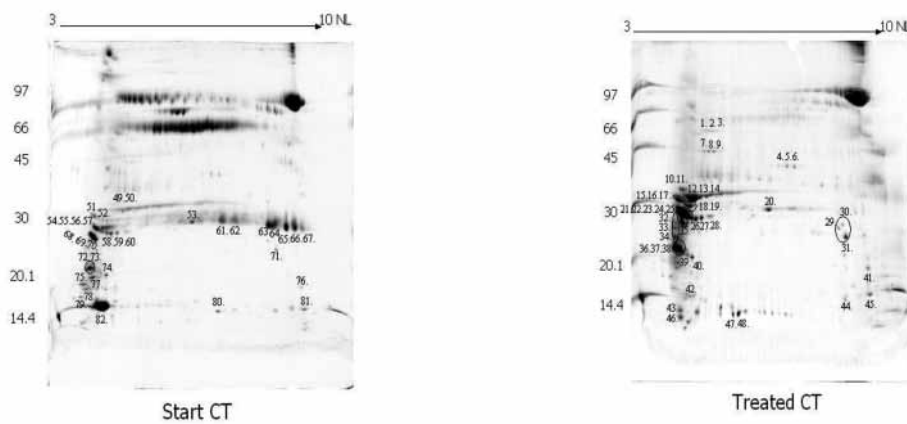


Fig. 1. 2D-Page map of 120 µg of protein in Start and Treated Term colostrum samples (CT).

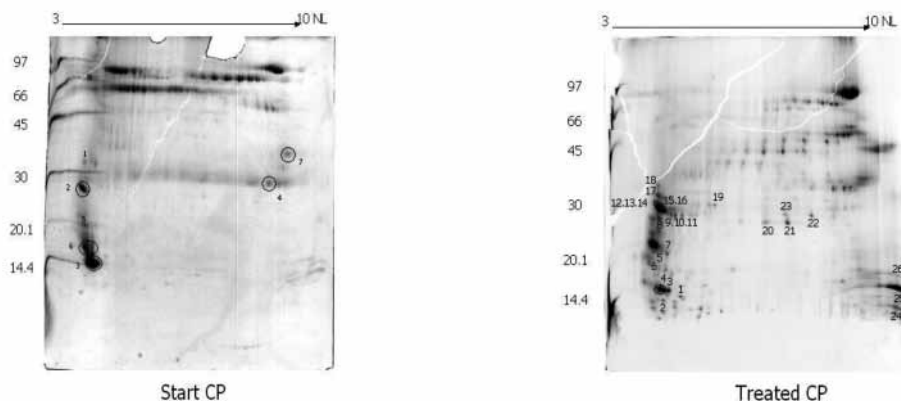


Fig. 1. 2D-Page map of 120 µg of protein in Start and Treated Preterm colostrum samples.

proteins present at high concentration (Proteominer Treatment).

Subsequently each sample were subjected to the steps of proteomic techniques (loading on gel, image acquisition by proteomic imaging system, in-gel tryptic digestion of proteins and protein identification by tandem mass spectrometry).

RESULTS

The most important result achieved in this study is the detection in human colostrum of one of the major allergens found in bovine milk: the intact isoform of bovine alpha-S1-casein (spot n.39 in Fig. 1).

Also in the preterm colostrum sample, most of the proteins identified were different isoforms of caseins not previously reported in literature. (spot n.1 and 18 in Fig. 2 and Tab. I)

The identification of alpha-S1-casein, the bovine allergenic protein in human colostrum, was possible due to the higher sensitivity, the specificity and the reproducibility of the proteomics technique: for the first time such protein was detected using mass spectrometry based methods, that are more reliable than immunoassay methods, used in the past.

DISCUSSION

The most relevant finding in this study was the detection of the intact bovine alpha-S1-casein in human colostrum. In previous studies α -lactoglobulin, (6) one of the major cow's milk allergen, was detected intact in human milk (6-8) in estimated concentrations of nanograms per litre during their peak concentration by

Spot no	Sample Name	Protein name	UniProtKB/Swiss-Prot name	UniProtKB /Swiss-Prot Accession number	Protein Mr	Protein p.I.	Protein sequence coverage	Peptide sequence
39	Treated CT	Alpha-S1-casein	CASA1_BOVIN	P02662	24570	4,98	14%	FFVAPFPEVFGK.E (38-49) EPMIGVNQELAYFYPELFR.Q (148-166)
1	Start CP	Alpha-S1-casein	CASA1_BOVIN	P02662	24570	4,98	17%	HQGLPQEVLENLLR.F (23-37) FFVAPFPEVFGK.E (38-49) YLGYLEQLLR.L (106-115)
18	Treated CP	Alpha-S1-casein	CASA1_BOVIN	P02662	24570	4,98	26%	HQGLPQEVLENLLR.F (23-37) FFVAPFPEVFGK.E (38-49) YLGYLEQLLR.L (106-115) EPMIGVNQELAYFYPELFR.Q (148-166)

Tab. 1. Identification of Apha-s1-casein from start CP, treated CP and Treated CT.

immunoassay based techniques. (6-8) Such techniques are very sensitive methods of detection of food allergens, yet they have the disadvantage of cross reacting with other proteins that could be present in the sample. (5) The problem of cross-reactivity is not negligible. Specific IgE (sIgE) for cow's milk proteins (CMP) have been reported to be present in blood sera of exclusively breast-fed infants: sIgE also for human milk whey proteins were found in the blood sera of atopic infants, and these sIgE strongly cross-reacted with the corresponding CMP (9). Moreover, immunoassay based methods focus on the detection of the intact protein and don't take into account the possible chemical modification or proteolytic digestion of proteins (5). On the contrary in our study, using ProteoMiner techniques, α -lactoglobulin was not detected in preterm or in term human colostrum. In fact mass spectrometry methods have high sensitivity, specificity and reproducibility and using a prefractionation step they increase the concentration of the most diluted proteins and simultaneously reduce the concentration of proteins present at high concentration. For this reason they can be considered more reliable than immunoassay methods in the detection of hidden allergens at very low concentration. According to our results bovine alpha-1-casein could be considered the cow's milk allergen that is readily secreted in human milk. As a consequence this protein could be a cause of sensitization to cow's milk in exclusively breastfed predisposed infants

It is interesting to note some differences observed in preterm colostrum compared to term colostrum samples,

in particular a higher concentration of bovine alpha-1-casein. A clear explanation of this observation is lacking. A possibility could be the different membrane permeability observed in mothers who delivered prematurely.

Further investigations are needed in order to clarify the clinical meaning of our findings in particular if alpha-1-casein has a major role in sensitization or in tolerance to cow's milk of exclusively breastfed predisposed infants. In fact the pathogenesis of food allergy is multifactorial, and it is also likely that the induction of oral tolerance is dependent on several conditions being met. For example, it might be important that food allergens be presented to the gastrointestinal tract in the context of breast milk, which contains numerous immunomodulatory and tolerogenic factors, such as oligosaccharides or TNF- α (10-12).

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