

Relationship between maternal- and fetal-specific IgE

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Abstract *Aim:* A positive correlation between maternal and cord-blood IgE levels is well documented for total IgEs, but not for specific IgEs. The difficulty in detecting specific cord-blood IgEs is due to their low concentrations, which hinder their dosage by low-sensitivity methods. The study aimed to correlate maternal and foetal specific IgEs against individual cow's milk proteins, detected by highly sensitive and specific techniques. *Methods:* Cow's milk specific IgE detection was performed by chemiluminescence on 52 specimens of maternal and cord blood after cow's milk protein separation by 1D and 2D gel electrophoresis. Cow's milk protein (CMP) antigens were identified by mass spectrometry techniques. *Results:* Specific IgEs for CMPs were found in 25/52 (48.1%) of maternal sera and in 19/52 (37%) of cord-blood sera. In order of decreasing frequency, the proteins found were BSA, IgG heavy chain, caseins and, in a single case, b-lactoglobulin. Positive cord-blood sera in all cases corresponded to a positive maternal result, and maternal and foetal immunoreactivity patterns were closely correlated. Moreover, in no case was there a positive cord-blood response with a negative maternal response. *Conclusion:* The study demonstrates a close relationship between maternal and cord-blood specific IgE patterns. The phenomenon observed could provide a model to elucidate the general production method of foetal IgEs, which might only be produced in the presence of both the corresponding maternal IgE and the related allergen.

Enrico Bertino¹, Carolina Bisson², Claudio Martano¹, Alessandra Coscia¹, Claudio Fabris¹, Giovanna Monti¹, Tiziana Testa² and Amedeo Conti³

¹Neonatal Unit – Department of Pediatric Sciences, University of Turin, Piazza Polonia 94, Turin, Italy,

²Institute of Science of Food Production – CNR, Turin, Italy, ³Food Allergy Service – Department of Pediatric Sciences, University of Turin, Italy

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Enrico Bertino, Neonatal Unit – Department of Pediatric Sciences, University of Turin, Piazza Polonia 94, Torino 10126

Tel.: 0039 011 3135775

Fax: 0039 011 3134612

E-mail: enrico.bertino@unito.it

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The fetus is thought to be able to produce immunoglobulin E (IgE) from as early as 11 wk of gestation (1) and, as cord-blood (CB) IgE are believed not to cross the placental barrier, they are thought to reflect fetal production.

A positive correlation between maternal and CB-IgE levels is well documented (2). This association may be explained by the report of maternal inheritance of atopic IgE responsiveness on chromosome 11q or by hitherto unidentified mechanisms, such as maternal factors affecting the fetal immune system.

However, studies that examined the relation between maternal and fetal IgE detected total IgE levels rather than those of specific IgE. The difficulty in detecting specific CB-IgE is because of their low concentrations, which

hinder their dosage by low-sensitivity methods. Methods that are more sensitive subsequently made it possible to quantify specific IgE in maternal and CB (1); however, these studies analyzed specific IgE for whole food (cow's milk and egg) and not for the individual proteins.

Mature cow's milk contains about 3.3% of proteins, of which about 80% consist of casein whereas the remaining 20% are serum albumin (MW 67.0), β -lactoglobulin (MW 18.3), α -lactalbumin (MW 14.2) and other low-abundance proteins. Cow's milk casein, one of the major allergens responsible for cow's milk allergy, is comprised of four proteins, α_{s1} -casein (MW 24.5), α_{s2} -casein (MW 26.1), β -casein (MW 26.6), and κ -casein (MW 19.0).

Our study aimed at detecting and comparing specific maternal and CB-IgE against individual cow's milk proteins (CMP) by proteomic techniques.

Methods

Subjects

The study included 52 healthy full-term newborns recruited in our neonatal unit by selecting those from non-atopic mothers and with family history negative for atopy. During pregnancy, cow's milk and derivatives had been unrestricted in the mothers' diet. Sera were collected at delivery by maternal venipuncture and aspiration of CB from the umbilical vein with a needle, after carefully cleaning the funiculus surface, through repeated blotting and rubbing with sterile gauze until the surface was completely dry. The medical staff involved in the research oversaw the accuracy of this procedure. Contamination by maternal blood was checked by measuring CB IgA levels; specimens with values above 10 ug/ml (3) were considered to be contaminated.

All mothers gave their informed consent. Ethic approval was obtained from the local Ethics Committee.

Preparation of CMP samples

Skimmed cow's milk was obtained by centrifugation (6000 *g*, 30 min, 10°C) and diluted 1:15 in cow's milk whey prepared by ultracentrifugation (189,000 *g*, 70 min, 6°C).

SDS-PAGE analysis and 2D gel electrophoresis

CMP were separated by SDS-PAGE as well as by 2D gel electrophoresis to identify bovine serum albumin (BSA) and/or IgG heavy chain and all components of the casein group. For SDS-PAGE analysis, samples were diluted (1:2) and boiled for 5 min in Laemmli buffer. Denatured proteins were separated on Mini-Protean (Bio-Rad, Hercules, CA, USA) using 12%T gels. For 2D gel electrophoresis separation, CMP were precipitated with a chloroform/methanol/water procedure, reduced for 1 h with 5 mm of tributylphosphine and alkylated for 2 h with 15 mm of iodoacetamide in rehydration buffer (7 m urea, 2 m thiourea, 4% CHAPS, 1% Triton X-100, 20 mm Tris and 0.2% Bio-Lytes 3–10 from Bio-Rad) prior to IEF. Reduced and alkylated samples were applied to 3–10 Ready Strip 7-cm (Bio-Rad) strips. Rehydrated strips were focused on the Protean IEF Cell (Bio-Rad)

for about 24 kVh, equilibrated in SDS-equilibration buffer and loaded onto SDS-PAGE minigel for further separation in the second dimension.

The proteins resolved by using both techniques were subsequently transferred to nitrocellulose membrane (0.2 μ m; Sigma, St Luis, MI, USA) by electroblotting on semi-dry transblot SD transfer cell (Bio-Rad) and stained with 0.2% Ponceau S (Amersham Life Science, San Francisco, CA, USA). Least molecular weight SDS-marker (Amersham) was used to determine molecular weight. Stained membranes were blocked by an incubation step in Tris Buffer Saline (TBS)/gelatine (0.5%).

Immunoblotting

For detection of specific IgEs directed against each CMP separated by 1D and/or 2D electrophoresis, immuno-labeling was performed with individual sera from all 104 subjects (52 mothers' blood and their 52 babies' CB). An IgE-free human serum was used as negative control. Blocked membranes were incubated in subjects' and control sera diluted 1:5 in Phosphate Tween Gelatine (PTG) buffer (0.02% gelatine, 0.25% IGEPAL CA-630 Sigma, in TBS) by means of gentle stirring at 37°C overnight. Immunoblots were then thoroughly rinsed with PTG and Horse Radish Peroxydase (HRP)-labeled anti-human IgE diluted 1:100,000 in PTG was applied for 1 h at 37°C. One blot was assayed with second antibody alone and showed no contribution from the conjugate (no aspecific reaction). Washed immunoblots were developed with Enhancement Chemiluminiscence Plus (ECL) + (Amersham Pharmacia) on autoradiographic films.

MALDI-MS analysis and MS/MS sequencing

To identify individual CMP, 2-Dimensional Electrophoresis (2-DE) gels were stained with Brilliant Blue G-Colloidal (Sigma). Spots containing proteins of interest were excised for in-gel trypsin digestion using a sequencing-grade modified trypsin (Promega, Madison, WI, USA). Protein identification by Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) and Tandem Mass Spectrometry (MS/MS) analysis were performed as described elsewhere (4).

Results

IgE specific for CMP were found in 25/52 (48.1%) of maternal sera and in 19/52 (37%) of

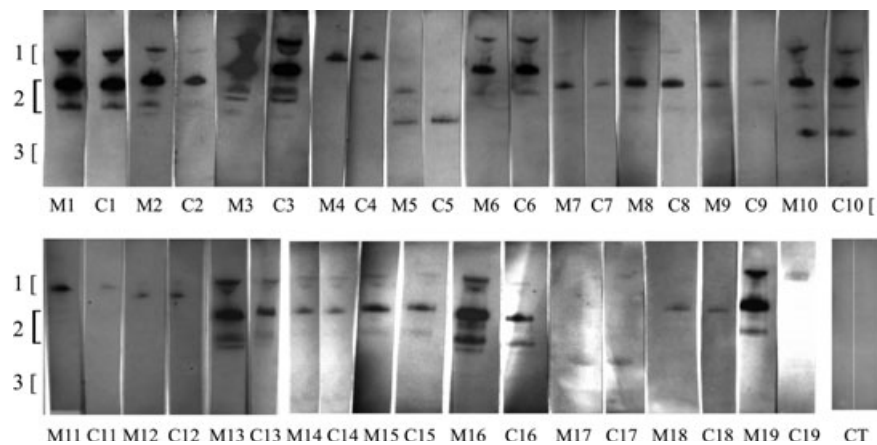


Fig. 1. Immuno-labeling for all 19 positive cord blood and relative maternal sera and the control subject. M, mother; C, cord blood; Ct, control. (1) Ig heavy chain, serum albumin zone; (2) casein zone; (3) α -lactalbumin, β -lactoglobulin zone.

CB sera. Fig. 1 shows SDS-PAGE and the results of the immuno-labeling for all 19 positive CB and relative maternal sera, and for the control subject. IgE-binding proteins were found at 60, 30, and 18 kDa, corresponding to BSA (and/or IgG heavy chain), the casein group and β -lactoglobulin, respectively.

BSA and/or IgG heavy chain were recognized by 17 CB sera (89%), caseins by 6 CB sera (31%), and β -lactoglobulin by 1 CB serum (5%). The separation of CMP by 2-DE was undertaken to discriminate between BSA and IgG and to separate all components of the casein group. Specific IgE against BSA were more frequently detected than those against bovine IgG (1:0.6) and, among the casein group, the reactivity was $\alpha_{s1} > \alpha_{s2} > \kappa$ -casein.

Fig. 2 shows the CMP separated by 2-DE and the result of the immuno-labeling for one of the 17 CB sera, with a positive signal at 60 kDa. It was thus possible to make a positive identifica-

tion of the specific IgE for bovine IgG heavy chain and, in this case, to assess the absence of the specific IgE for BSA.

Notably, positive CB sera in all cases corresponded to a positive maternal result and the comparison between maternal and fetal immuno-reactivity showed strict pattern correlation. Moreover, in no case did a positive CB response correspond to a negative maternal response. Of the 33 negative CB responses, 6 corresponded to positive and 27 to negative maternal responses. None of the 52 infants had developed clinical signs of CMP allergy/intolerance by 1 yr of age.

Discussion

This study for the first time applies a proteomic approach to identifying individual CMP against which maternal and cord IgE are directed. This method allowed us to detect IgE against CMP in 25/52 mothers, though without allergic

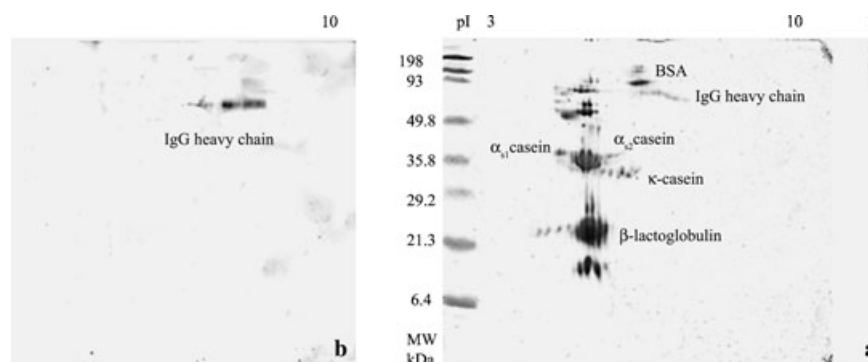


Fig. 2. Panel A: 2D electrophoretic separation of cow's milk proteins stained with Coomassie blue. Identification of labeled spot was achieved by MALDI-TOF after trypsin 'in-gel' digestion. Panel B: Cord blood no. 4 immunoreactivity against cow's milk proteins. The IgE of this subject only reacted with bovine IgG heavy chain.

symptoms. The presence of detectable IgE in asymptomatic subjects has frequently been reported (5).

The high percentage of cases in which specific CB IgE against CMP were detected (19/52 → 37%) is also in agreement with data from studies using sensitive methods that report incidences ranging from 9–11% (6) to 43.8% (1).

The fact that none of the 52 infants developed clinical signs of CMP allergy at 1 yr of age, despite statistics in literature showing an approximate incidence of 2–3% (7) confirms the hypothesis that these responses may be normal immunologica phenomena, without being related to the subsequent development of food allergy (8).

The most surprising result is the close relationship between maternal and CB IgE: (i) all CB samples from newborns of negative mothers were also found to be negative; (ii) all cases of positive CB samples were associated to positive maternal IgE; (iii) there was close correspondence between maternal and CB patterns of IgE specific against individual CMP.

These observations may be interpreted in various ways. The first hypothesis is that the IgE we found in the CB are the consequence of maternal blood contamination of CB samples during delivery. However, maternal blood contamination can be ruled out, as we took the CB samples by direct needle aspiration; this method has been shown to be the best blood-sampling technique to avoid contamination, on a par with neonatal capillary collection at days 4–5 (9). We further increased the reliability of the sampling method by carefully cleaning the funiculus surface before puncturing the vein.

Genetic factors (9) may also be a cause of maternal influence on CB-IgE levels, but cannot of themselves explain the close antigen-specific correspondence of maternal and CB IgE found in our study. Similarly, transplacental passage of maternal cytokines cannot be the sole factor, as these do not condition an antigen-specific immune response from the fetus.

A further hypothesis might be that the IgE we found in the CB are of maternal origin. The placental barrier is believed to prevent all immunoglobulin isotypes except IgG being transported from mother to fetus (10). However, human IgE administered to pregnant animals has been reported to pass from mother to fetus at a fractional rate similar to that of albumin. These observations open the possibility that, at least in animals, IgE might be transported across the placental barrier, although not with efficacy similar to that of IgG (2).

A more likely hypothesis to explain our observations is the possibility of dia-amniotic fluid transfer and fetal swallowing of maternal IgE (11–13). Maternal IgE might reach the amniotic liquid through the deciduous vascular system and then be swallowed by the fetus. The IgE would then link to either high- or low-affinity IgE-receptors on dendritic cells and B-cells, respectively, of the gut-associated lymphoid tissue (GALT). The presence of specific IgE in the fetal gut-associated immune system would cause a higher concentration of the antigen, which would increase the possibility of fetal sensitization, enabling antigen presentation to occur even with quantities of antigen that are 100–1000 times lower than those necessary in the absence of IgE. CMP, whose transplacental passage and passage into the amniotic liquid (11) are well documented, would then come into contact with the maternal CMP-specific IgE linked to the fetal GALT cells, causing production, in the fetus, of the specific IgE directed against the same antigens against which the maternal IgE are directed. The fact that in this study the mothers' diet during pregnancy included cow's milk and derivatives is compatible with this hypothesis.

The maternal/fetal correlation of the specific CMP IgE pattern observed could help to clarify the general production method of fetal IgE, the production of which might only occur in the presence of both the corresponding maternal IgE and the related allergens.

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