

Original article

Cow's milk-specific cellular and humoral immune responses and atopy skin symptoms in infants from atopic families fed a partially (pHF) or extensively (eHF) hydrolyzed infant formula

Background: Hydrolyzed milk formulas are recommended to feed infants at high risk of atopy if breast-feeding is not possible. We studied the specific cellular and humoral immune response to cow's milk proteins and occurrence of atopic dermatitis under different feeding regimens: two hydrolyzed infant milk formulas (partially [pHF] and extensively hydrolyzed [eHF]) and under exclusive breast-feeding (BF).

Methods: Seventy-two infants from families with atopic symptoms were randomized in the pHF and eHF groups, respectively. At 6 and 12 months of age, peripheral blood mononuclear cell proliferation along with specific IgG and IgE to cow's milk proteins was determined in infants fed pHF or eHF, respectively, and those who had not yet received any formula at 6 months of age (BF). Cases of atopic dermatitis were recorded throughout the first 12 months of life, and their severity was evaluated with SCORAD points.

Results: A significantly decreased proliferation to cow's milk caseins was found in the pHF group compared to the exclusively breast-fed group. Medians of stimulation indexes for CAS at 6 months were as follows: pHF 1.18; $n=24$; BF 1.70; $n=24$ ($P=0.033$, Mann-Whitney U-test). Higher levels of plasma IgG antibodies to BCAS were found in infants fed pHF than in those fed eHF at 12 months. Optical density (OD): (25th percentile; median; 75th percentile): pHF: 0.00; 0.14; 0.38; $n=30$; eHF: 0.00; 0.03; 0.14; $n=28$; $P=0.089$, Mann-Whitney U-test. Cow's milk-specific IgE was detected at 6 months as follows: BF: 3 of 24; eHF: 2 of 21; pHF: 0 of 23. The number of cases of atopic dermatitis and their severity did not differ among the groups during the first 12 months.

Conclusions: Feeding pHF appears to suppress cow's milk-specific cellular responses and stimulate specific IgG production. Specific IgE sensitization can occur also with breast-feeding.

I. Nentwich¹, E. Michková²,
J. Nevoral², R. Urbanek¹,
Z. Szépfalusi¹

¹Department of Pediatrics, University of Vienna, Vienna, Austria; ²First Pediatric Clinic, Charles University, Prague, Czech Republic

Key words: atopy; breast-feeding; casein; cellular immune; cow's milk; IgE; IgG; infant formulas; β -lactoglobulin.

Zsolt Szépfalusi, MD
Clinic of Pediatrics
Vienna University
Währinger Gürtel 18–20
A-1090 Vienna
Austria

Accepted for publication 12 July 2001

Hydrolyzed infant formulas were developed several decades ago to meet the nutritional requirements predominantly of infants allergic to cow's milk and those with malabsorption. As a result of extensive protein hydrolysis, they contain a mixture of amino acids or low-molecular-weight peptides with very low residual antigenicity and are used mostly in therapeutic indications. Due to the high hydrolysis of the protein source, they usually have a bitter taste.

In the late 1980s, partially hydrolyzed infant formulas

(pHF) were introduced with a lower degree of hydrolysis, better palatability, and lower price. They were intended as a part of a strategy to prevent the development of atopic symptoms in infants with high risk of atopy when breast-feeding was not possible. In the early 1990s, studies on the atopy preventive effects of partial hydrolysates compared to unhydrolyzed milk formulas were done (1). However, they failed to demonstrate the preventive effect of pHF during the first years of life. Most studies even lack proper definition of atopic disease and atopic heredity.

The use of extensively hydrolyzed infant formulas (eHF) for atopy prevention in infants with a familial history of atopy was considered and proved to be effective in studies comparing preventive effects of eHF with unhydrolyzed milk formulas (2–4). Almost all the studies have proved a limited prophylactic effect,

Abbreviations. eHF: extensively hydrolyzed formula; pHF: partially hydrolyzed formula; BF: exclusive breast-feeding; HA: hypoallergenic; CMF: cow's milk-based infant formula; SCORAD: scoring of atopic dermatitis; AD: atopic dermatitis; IgG: immunoglobulin G; IgE: immunoglobulin E; SI: stimulation index; PBMC: peripheral blood mononuclear cells; cpm: counts per minute.

indicating that the use of hypoallergenic formulas can at least postpone the onset of atopic symptoms during the first 3 years of life and/or alleviate them. A study by Zeiger & Heller (5), however, has shown a preventive effect only for food allergy up to the age of 2 years.

Nevertheless, the question of whether eHF grants more effective and longer protection against the development of atopy symptoms and sensitization to cow's milk proteins than pHF has remained unsolved. Even the recommendations of pediatrics boards in Europe are contradictory. The European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) issued a position paper on the use of allergen-reduced infant formulas (6), claiming that the use of pHF instead of eHF is satisfactory for the prevention of atopy symptoms when breast-feeding is not possible. However, in a position paper (7), the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) recommends only eHF for atopy prevention in infants with a high risk of atopy.

Several studies published so far compare eHF with pHF in their atopy-preventive effects. Oldæus et al. (8) used two hydrolysates differing not only in the degree of hydrolysis but also in the protein source used (whey vs casein hydrolysates). Recently, a large prospective, randomized, placebo-controlled, double-blind study comparing pHF with two kinds of eHF was published (9). Both studies demonstrate that eHF is more effective in the prophylaxis of atopy symptoms, especially atopic dermatitis (AD). Comparative studies on the cellular and humoral immune response in infants receiving two different formulas on the same protein basis (cow's milk whey protein hydrolysate), and differing only in their degree of hydrolysis, together with their atopy preventive effects, have not been published yet.

The aim of our study was to compare

- 1) the antigen-specific reactivity of peripheral blood mononuclear cells to cow's milk proteins
- 2) the plasma levels of cow's milk-specific IgE and IgG antibodies as well as the total IgE antibodies
- 3) the development of atopic skin symptoms

between groups of infants from families with atopic history, who were fed, respectively, an extensive hydrolyzed whey formula or a partially hydrolyzed whey formula, or were exclusively breast-fed, throughout the first 12 months of life.

Material and methods

Proband recruitment and group randomization

Pregnant women living in the urban region of Prague, Czech Republic, who themselves, their infants, or their husbands had been treated at an allergology or dermatology outpatient department during the years 1994–6 were invited to participate in the study. This was done by means of an invitation leaflet, which had been sent to these departments.

During a telephone registration of mothers at the First Clinic of Pediatrics in Prague-Motol, Department of Gastroenterology and Nutrition, they were briefly questioned about their own and their relatives' family history of atopy, and put on a list where odd numbers indicated the pHF group and even ones the eHF group, respectively. They were advised to deliver at either of two obstetric clinics which participated in our study (Department of Obstetrics, Prague-Motol, and Department of Obstetrics, Prague-Londýnská), and they had been previously supplied with both hydrolysates used in our study. As allergen elimination diets during pregnancy have not shown any prophylactic effect (10, 11), no dietetic intervention was made during pregnancy. The mothers were encouraged to breast-feed for at least 6 months after delivery while avoiding fresh milk and some highly allergenic foods such as nuts and honey in their diet during lactation, and were recommended to eat dairy products daily and fish approximately once every 2 weeks. If breast-feeding was not possible or sufficient, the respective hypoallergenic formula according to the randomization was recommended. Moreover, general atopy prevention measures such as a ban of smoking and elimination of pets in the family were advised. Both parents were invited to the first clinical visit with the infant 2 months after birth. Finally, both obstetric clinics involved and the family pediatrician, if known, were notified of the mother's name and birth term, and the respective hydrolysate group the mother had been assigned to. A nonstop dispensing service of the hydrolysates at our pediatrics gastroenterology ward was organized, where parents received the hypoallergenic formulas.

At admittance to the obstetric clinics, the mothers were notified once more about participating in the study and their hydrolysate group. Before the onset of lactation, glucose solution or, if necessary, the respective hydrolysate formula was fed to the neonates. After this time, the hydrolyzed formula was administered only if breast-feeding was not possible or was insufficient.

Approximately 6 weeks after birth, the mothers made an appointment by telephone for the first visit to our outpatient department for high risk-of-allergy infants.

Parental history of atopy

The first detailed family history of atopy was not taken until 2 months after birth. History of atopic symptoms in parents and siblings, if applicable, was taken from both parents by means of a standard questionnaire. Questions about symptoms suggestive of bronchial asthma covered occurrence of attacks of cough and/or wheezy breathing and/or dyspnea during the pollen season, in the night, or after contact with animals. For seasonal/perennial rhinitis (rhinoconjunctivitis), the questions included occurrence of nasal pruritus and/or obstruction and/or discharge and/or eye edema or pruritus during the pollen season, or in the night or after contact with animals. For AD, the questions covered occurrence of chronic itching skin exanthema, in particular in the flexural folds. Furthermore the relatives were asked what diagnosis had been registered by the allergologist/dermatologist and whether they underwent skin prick testing. No other investigations were done in relatives.

If they had symptoms of atopy for at least 24 months and were registered for any of the three diagnoses stated above, they were classified as atopic. If they had never had symptoms, they were classified as nonatopic.

In addition, smoking and pets kept in the household were documented.

High-risk group definitions

Infants for whom at least one first-degree relative (father, mother, or sibling) was classified as atopic according to the criteria described above, who were born at term (38–42 weeks gestational age) with a birth weight over 2500 g, and who had no perinatal disorder (grave jaundice, asphyxia, etc.) were included for further follow-up.

Clinical follow-up

The clinical visits were organized as follows: in the first room, the pediatrician, the organizer of the study, who knew the randomization protocol, took the history, gave nutritional recommendations, and dispensed the formulas, if applicable. In the adjoining room, another pediatrician, the same at all times, examined the infants without knowing the formula used, and ordered treatment, if necessary.

At the 2-month-visit, the perinatal history and the birth weight were recorded, and the parents were asked whether the infant received any formula at the neonatal unit. We then asked whether the mother breast-fed and if she drank fresh cow's milk and ate dairy products. If so, we documented the amount of fresh milk received per day. This inquiry was done only once at the 2-month-visit. If formula had already been given before the first visit, the time of the first formula introduction, the time of the last breast-feeding, and the amount of formula fed were recorded. If breast-feeding was not possible or sufficient, the respective hypoallergenic formula was prescribed according to the randomization protocol.

At this time, the parents received a follow-up booklet which included, besides information about the study protocol, also record charts for the period between two visits, where they recorded the time of first formula introduction and last breast-feeding, and the amount of formula fed. Furthermore, they recorded the time and duration of skin symptoms in the charts and inscribed their distribution and character into a predrawn silhouette picture.

The pediatric examination consisted of weighing on always the same scale and physical examination. The results of the examination were inscribed in the routine patient record and doubled in an extra study protocol.

Further clinical visits were organized at the ages of 4, 6, 9, and 12 months. Starting with the 4th month of age, the follow-up booklet was reviewed and nutrition evaluated. The duration of exclusive breast-feeding was calculated as the date of first formula introduction (except when bottle-fed before lactation had started) minus the date of birth. The duration of milk feeding was calculated as the date of the respective visit minus the date of first formula introduction. The duration of mixed feeding (breast-feeding and formula) was calculated as the date of the last breast-feeding minus the date of the first formula feeding. If the date of the last feeding had preceded the date of the respective visit, the duration of the mixed feeding was equal to the duration of the formula feeding. At all visits, general nutritional advising and physical examination were done. In addition, at the 4-month visit, the parents were instructed thoroughly about the supplementary food introduction protocol and were given written information on when, which, and how supplementary food should be introduced to the infants. At 6 and 12 months of age, venous blood was drawn from the infants (see below).

Definition of the feeding groups. Infants who received only mother's milk during the period before examination (with the exception of feeding the respective hydrolysate formula before onset of lactation) were classified as exclusively breast-fed. Infants who received the respective hypoallergenic formula according to the randomization protocol for a period of at least 7 days before examination were classified as formula fed. The period of milk feeding for a minimum of 7 days should be sufficient to mount a specific immune response in primed individuals.

Dropouts. If parents stopped attending the regular clinical visits at any time from the infant's birth to the age of 12 months, they were classified in dropout group I. No dropout analysis was done in this group. Parents whose infants received more than one and formula other than that which had been randomly assigned to

them were classified in dropout group II. The reason was investigated at the next visit. Although nutritional advising was given in dropout group II, no data were evaluated.

Supplementary food

Because the introduction of supplementary food significantly affects the manifestation of atopic symptoms in high-risk infants (12, 13), a standardized supplementary food protocol was used in all infants. It included introduction of the same sort of supplementary food (carrot, potato, beef) at the standard time (6 months of age) by the standard manner (three-step protocol: first week, stewed carrot; second week, carrots and potato; third week, carrot with potato and beef). After this introductory protocol, well-known nutritional allergens (cow's milk and milk products, eggs, fish, nuts, and highly allergenic vegetables and fruits) were eliminated until the age of 12 months.

Skin symptoms

Skin symptoms were documented by parents continuously in the follow-up protocols, but not evaluated by the pediatricians until the end of the clinical follow-up. At each visit, skin symptoms were drawn in a specific SCORAD record. For the diagnosis of AD, we applied criteria also used by Halken et al. Briefly, atopic eczema was diagnosed if physical examination revealed areas of scaly, erythematous, and itchy eczematous rash, primarily of the face, the scalp, and the flexural folds. Only eczema with at least two locations in typical areas relapsing with a duration of at least 3 months was recorded. For scoring the severity of the lesions, we applied the standard SCORAD system described elsewhere (14), the results being shown as SCORAD index values. SCORAD values of only those infants in which AD was diagnosed are presented under Results. The development of the SCORAD index during the evaluation period was influenced by application of topical steroids in some cases. This was recorded, and the SCORAD values of those patients were excluded from statistical analyses.

Hypoallergenic formulas

As already stated, feeding hypoallergenic formulas was recommended only when breast-feeding was not possible or sufficient.

The partially hydrolyzed infant formula (Beba H.A.[®], Nestlé, Denmark) used in our study is based on partially hydrolyzed bovine whey with relatively high residual antigenicity (15), while the extensive hydrolysate (Hipp H.A.[®], Hipp GmbH, Gmunden, Austria) is an infant hypoallergenic formula based on extensively hydrolyzed bovine whey with low residual antigenicity. The molecular weight profiles of both formulas used in our study have previously been evaluated by gel permeation chromatography under uniform conditions (16). According to the manufacturers, the protein/peptide component of the Beba H.A. used in our study is identical to that of NAN H.A. distributed by the same manufacturer. Moreover, the protein/peptide component of the Hipp H.A. used in our study is identical to that of Nutrilon Pepti[®].

Ethical considerations

Parents were instructed about the hypoallergenic formulas used. At the first clinical visit, they signed a written, informed consent form covering blood sampling and analyses.

Both H.A. formulas were formerly tested and are commercially available in Europe. The formulas were recommended only if breast-feeding was not possible, and the importance of breast-feeding was always stressed. The formulas used were bought from the companies and dispensed to the parents free of charge. The study was approved by the local ethics committee of the Prague-Motol Hospital.

Laboratory investigations

Blood sampling. Peripheral venous blood was taken from infants at the age of 6 and 12 months into heparinized glass tubes (Vacutainer, Becton-Dickinson) and centrifuged to obtain plasma for antibody analyses. Blood cells were then separated as stated below.

Cow's milk-specific PBMC proliferation. To measure PBMC proliferation in response to cow's milk protein stimulation, a standard method was used, as described in the literature (17, 18). Briefly, mononuclear cells from heparinized peripheral blood were isolated by means of gradient centrifugation (Ficoll Paque, Pharmacia Uppsala, Sweden). After washing, 100 000 mononuclear cells in 100 µl cell culture medium per well were incubated with the respective antigens for 5 days. The following milk proteins were used as antigens in a concentration of 25 µg/ml: α -lactalbumin (ALA); β -lactoglobulin (BLG); whole casein (CAS) and its subunits α -, β -, and κ -casein (ACAS, BCAS, KCAS); and bovine serum albumin (BSA). After 5 days, ^3H -labeled thymidine (Amersham, UK) was incorporated for 12 h, and scintillation was measured by a liquid scintillation detector. The results are expressed as the stimulation index (SI) calculated from the formula: SI = sample scintillation [cpm]/blank scintillation (medium control) [cpm]. As a positive control, human recombinant interleukin-2 (Behring, Germany) was used (20 U/ml/well).

Antibody assays

Cow's milk-specific IgG antibodies. To analyze levels of cow's milk-specific IgG, we used a modified immunoblotting method already described for IgE detection (19). Unhydrolyzed cow's milk-based infant formula (Aptamil 1, Milupa, Germany) was defatted by centrifugation and placed onto SDS-polyacrylamide gel (Excel Gel® 15%, Pharmacia Biotech, Uppsala, Sweden) at a concentration of 10 µg protein/lane. After resolving, proteins were transferred onto 0.2 µm nitrocellulose membrane (Transferblot® Bio-Rad, Hercules, CA, USA), which was blocked with 5% human serum albumin in Tris-buffered saline (TBS), cut into strips, and incubated with infants' plasma samples (dilution 1:500) for 2 h. IgG antibodies were detected with rabbit antihuman IgG (Dako, Denmark) as the primary antibody, and donkey antirabbit Ig labeled with ^{125}I (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as the secondary antibody. After washing, the strips were exposed to a sensitive autoradiographic film (Hyperfilm MP, Amersham Pharmacia Biotech AB, Uppsala, Sweden) at -70°C for 5 days. Quantification was performed by densitometry (Elscrip 400, Hirschmann, Germany), the results being expressed as optical density (OD).

Cow's milk-specific IgE antibodies. Basically, the same method as for IgG detection was used for specific IgE determination with the following modifications: patient plasma diluted 1:5 was incubated for 16 h at 4°C and then washed off, and bound IgE was detected with ^{125}I -labeled rabbit antihuman IgE (Kabi Pharmacia, Sweden). Strips were exposed to a sensitive autoradiographic film (Hyperfilm MP, Amersham Pharmacia Biotech AB, Uppsala, Sweden) at -70°C for 28 days. Any detectable bands were considered positive.

Total IgE antibodies. Total IgE antibody concentrations were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) (Kallestad, Sanofi Diagnostics, Pasteur, USA). The sensitivity of the assay was 0.5 kU/l.

Data analysis

All data were collected in a database (Microsoft Access 97®), and the appropriate variables transferred to the statistic and graphic software GraphPad Prism®, Version 3.0 (GraphPad Software, San Diego, CA, USA). For continuous variables, descriptive statistics were done for each data set, and the normality test was run on the normal distribution. If the data sets did not significantly differ from the normal distribution on the 95% significance level, parametric tests (unpaired *t*-test) were applied. If the variable was not normally distributed, the data were logarithmically transformed, the normality was retested, and a parametric test was used. If this transformation was not possible or not suitable (e.g., many values were equal to zero), nonparametric tests were used (the Mann-Whitney U-test). Power calculations were done with GraphPad StatMate®, Version 1.01 (GraphPad Software, San Diego, CA, USA).

Results

Feeding group distribution

Randomization results. Seventy-three families were registered by telephone before birth (37 families assigned to the pHF group and 36 to the eHF group).

At the first clinical visit (2 months after birth), 72 babies continued the study (one family from the eHF group did not attend the first visit). As a detailed history of atopy was taken at the age of 2 months after birth, the data of only 72 infants are given. According to the randomization protocol, the pHF consisted of 37 infants (15 male, 22 female), and the eHF of 35 infants (17 male, 18 female).

Family history of atopy. In the pHF group, out of 37 families, 24 mothers were atopic and 13 nonatopic. Out of 24 atopic mothers, 16 were skin prick test positive for inhalant allergens, and eight negative or not tested. Fourteen fathers were atopic, and 23 nonatopic. Out of 14 atopic fathers, 12 were skin prick test positive for inhalant allergens, and two negative or not tested. Out of 25 families with siblings, 22 were atopic, and three nonatopic. Out of 22 atopic siblings, 10 were skin prick test positive for inhalant allergens, and 12 negative or not tested. Atopy only in the mother was present in 18 families; atopy only in the father in eight families. Both atopic mothers and fathers were found in six families. Atopy only in siblings was found in six families.

In the eHF group, out of 35 families, 22 mothers were atopic and 13 nonatopic. Out of 22 atopic mothers, 14 were skin prick test positive for inhalant allergens and eight negative or not tested. Nineteen fathers were atopic and 16 nonatopic. Out of 19 atopic fathers, 14 were skin prick test positive for inhalant allergens and five negative or not tested. Of 27 families with siblings, 22 were atopic and five nonatopic. Out of 22 atopic siblings, 12 were skin prick test positive for inhalant allergens and 10 negative or not tested. Atopy only in the mother was found in 14 families; atopy only in the

father in 11 families. Both atopic mothers and fathers were found in eight families. Atopy only in siblings was found in two families.

Smoking in the family. In the pHF group, there was no smoking in 26 families, the father alone smoked in 10 families, the mother alone smoked in one family, and both parents smoked in no family. In the eHF group, there was no smoking in 23 families, the father alone smoked in 11 families, the mother alone smoked in no family, and both parents smoked in one family.

Domestic animals. In the pHF group, there were no pets in 33 families, a cat in two families, a dog in one family, and a budgerigar in one family. In the eHF group, there were no pets in 33 families, a dog in one family, and a budgerigar in one family.

Feeding at the obstetric department. According to the prenatal randomization, out of the pHF group, 32 infants were exclusively breast-fed immediately after birth, and five received pHF formula before lactation began. Out of the eHF group, 28 infants were exclusively breast-fed immediately after birth, and seven received eHF formula before lactation began. None of the infants received a “wrong” formula.

Cow's milk ingestion during lactation. At the 2-month visit, 26 mothers reported drinking cow's milk with the mean amount of 0.45 and SD 0.32 l/day. The pHF group mothers ($n=12$), did not significantly (95% level) differ in the reported amount of cow's milk drunk from the eHF group mothers ($n=14$) (mean; SD in l/day: pHF 0.32; 0.15 and eHF 0.56; 0.39, $P=0.054$; unpaired t -test). Forty-six mothers reported abstinence (pHF 25, eHF 21) from drinking cow's milk. All mothers ate dairy products (yoghurt, cheese, etc.) daily.

Two-month feeding groups. Forty-nine infants were exclusively breast-fed at the age of 2 months (23 of them belonged to the pHF and 26 to the eHF groups). Twelve received pHF and nine eHF, one infant from the pHF group could not be assigned to any group (formula feeding was too short), and one infant (from pHF) dropped out (dropout group II), as the parents refused to feed the hydrolysate formula.

Out of 49 breast-feeding mothers at the 2-month visit, 17 (pHF 9, eHF 8) reported drinking cow's milk, and 32 (pHF 14, eHF 18) reported abstinence. The pHF group mothers ($n=9$) differed significantly from the eHF group mothers ($n=8$) in the amount of milk drunk (mean; SD in l/day: pHF 0.34; 0.16 and eHF: 0.68; 0.40. $P=0.033$; unpaired t -test).

Two-month formula-feeding pattern. In the pHF group, 12 infants received pHF with the mean formula-feeding period of 42.2 days (SD 19.2; extreme values 11.0–68.0 days), the mean exclusive breast-feeding period of 20.3 days (SD 17.3; 0.0–48.0 days), and the mean partial formula-feeding period of 39.9 days (SD 18.9; 11.0–68.0 days).

In the eHF group, nine infants received eHF with the mean formula-feeding period of 44.7 days (SD 17.0; extreme values 19.0–67.0 days). No significant difference was detected compared to the pHF group (P value 0.760; 95% CI –14.37 to 19.37; unpaired t -test). The mean exclusive breast-feeding period of 160 days (SD 159; 0.0–430 days) did not significantly differ from the pHF group (P value 0.571; 95% CI –19.69 to 11.19; unpaired t -test), nor did the mean partial formula-feeding period of 36.7 days (SD 17.9; 17.0–64.0 days; P value 0.694; 95% CI –20.30 to 13.80; unpaired t -test).

4-month-feeding groups. Thirty-two infants were exclusively breast-fed at the age of 4 months (14 of them belonged to the pHF and 18 to the eHF groups). Twenty-two received pHF and 15 eHF, one infant from the eHF group could not be assigned to any group (formula feeding was too short), and two infants dropped out (dropout group II), one from the pHF group (parental refusal), and one from the eHF group (poor acceptance of the formula).

Out of 32 breast-feeding mothers at the 4-month visit, 11 (pHF 5, eHF 6) reported drinking cow's milk and 21 (pHF 9, eHF 12) reported abstinence. The groups of milk-drinking mothers were too low to compare any difference.

Four-month formula-feeding pattern. In the pHF group, 22 infants received pHF with the mean formula-feeding period of 74.0 days (SD 41.0; extreme values 7.0–146.0 days), the mean exclusive breast-feeding period of 48.6 days (SD 36.8; 0.0–111.0 days), and the mean partial formula-feeding period of 56.4 days (SD 39.0; 3.0–146.0 days).

In the eHF group, 15 infants received eHF with the mean formula-feeding period of 79.1 days (SD 34.5; extreme values 24.0–133.0 days). No significant difference was detected compared to the pHF group (P value 0.690; 95% CI –21.02 to 31.37; unpaired t -test). The mean exclusive breast-feeding period of 47.3 days (SD 42.6; 0.0–121.0 days) did not significantly differ from the pHF group (P value 0.924; 95% CI –27.93 to 25.42; unpaired t -test), nor did the mean partial formula-feeding period of 67.3 days (SD 36.5; 3.0–114.0 days) (P value 0.398; 95% CI –17.97 to 36.77; unpaired t -test).

Six-month feeding groups. Twenty-four infants were exclusively breast-fed at the age of 6 months (11 of

them belonged to the pHF and 13 to the eHF groups). Twenty-four received pHF and 21 eHF. Three infants dropped out (dropout group II), two from the pHF group (both parental noncompliance) and one from the eHF (poor acceptance of the hydrolyzed formula).

Out of 24 breast-feeding mothers at the 6-month visit, nine (pHF 5, eHF 4) reported drinking cow's milk and 15 (pHF 6, eHF 9) reported abstinence. The groups of milk-drinking mothers were too low to compare any difference.

Six-month formula-feeding pattern. In the pHF group, 24 infants received pHF with the mean formula-feeding period of 127.9 days (SD 47.5; extreme values 33.0–195.0 days), the mean exclusive breast-feeding period of 62.4 days (SD 48.9; 0.0–158.0 days), and the mean partial formula-feeding period of 106.5 days (SD 47.6; 21.0–195.0 days).

In the eHF group, 21 infants received eHF with the mean formula-feeding period of 111.1 days (SD 59.6; extreme values 6.0–189.0 days). No significant difference was detected compared to the pHF group (P value 0.301; 95% CI – 48.99 to 15.52; unpaired t -test). The mean exclusively breast-feeding period of 77.4 days (SD 61.7; 0.0–182.0 days) did not significantly differ from the pHF group (P value 0.369; 95% CI – 18.32 to 48.24; unpaired t -test), nor did the mean partial formula-feeding period of 93.2 days (SD 64.5; 5.0–176.0 days; P value 0.433; 95% CI – 47.10 to 20.57; unpaired t -test).

As the 6-month feeding groups are of particular importance with respect to their specific cellular and humoral immune response to the cow's milk antigens tested, further distribution description is given.

Sex distribution within the 6-month groups. The breast-fed group comprised 10 boys and 14 girls (pHF: 10 boys, 14 girls; eHF: 10 boys, 11 girls). The dropout group II comprised two boys and one girl.

Family history of atopy within 6-month groups. In the pHF group, there were four infants with double parental positive and 20 with single-positive (12 mother, five father, and three sibling) family history of atopy. In the BF group, there were four infants with double parental positive history, and 20 with single positive (eight mother, nine father, and three sibling). In the eHF group, there were five infants with double-positive history, and 15 with a single-positive history (11 mother, four father, and one sibling). In the dropout II group, there was one infant with double parental positive history, and two with single positive (one mother, one father).

Smoking in the family within 6-month groups. Out of the breast-fed group ($n=24$), smoking in the

household was reported in four infants, whereas no smoking was reported in 20 families. Further results were as follows: feeding group: number of households with smoking/no smoking – pHF: 9/15; eHF: 9/12; dropout II: 1/2. We could not find any significant difference in the proportions of smoking/no smoking households among the particular feeding groups (Fisher's exact test).

Taken together, in the bottle-fed groups (pHF + eHF + dropout II), smoking was reported in 19 cases and no smoking in 29 cases. The difference between the breast-fed and the bottle-fed groups in the above proportions was near significance ($P=0.062$; Fisher's exact test). The test gives 90% power to detect the smallest interesting difference of 0.43 between proportions. The mean duration of exclusive breast-feeding at the 6-month visit was insignificantly lower in infants from smoker than from nonsmoker families (mean duration in days; SD: smokers 56.63; 51.45; $n=19$ and 74.59; 56.50; $n=29$; $P=0.271$; unpaired t -test). The test gives 50% power to detect the smallest interesting difference of 30 days when comparing means with unpaired t -test for the given SD.

Domestic animals within 6-month groups. Out of the pHF group, 22 families kept no pets, one family kept a dog, and one family kept a cat. Out of the BF group, 22 families kept no pets, and two families kept cats. Out of the eHF group, 19 families kept no pets, one family kept a dog, and one family kept a budgerigar. Out of the dropout II group, all three families kept no pets.

Nine-month feeding groups. No infants were exclusively breast-fed at the age of 9 months. Thirty-one received pHF and 27 eHF. Three infants (all eHF) received only mother's milk with supplementary food but no formula (evening milk pap was made of mother's milk), and two (pHF) could not be assigned due to a too short formula feeding. Three infants (1 pHF, 2 eHF) belonged to the dropout group I, whereas six (3 pHF, 3 eHF) dropped out to the dropout group II (feeding unhydrolyzed formula due to parental noncompliance or poor acceptance of the hydrolyzed formula).

Nine-month formula feeding pattern. pHF group: 31 infants received pHF with the mean formula-feeding period of 172.5 days (SD 84.0; extreme values 31.0–299.0 days), the mean breast-feeding period of 107.6 days (SD 79.9; 0.0–254.0 days), and the mean partial formula-feeding period of 146.8 days (SD 74.1; 31.0–267.0 days).

In the eHF group, 27 infants received eHF with the mean formula-feeding period of 160.6 days (SD 81.0; extreme values 10.0–273.0 days). No significant differ-

ence was detected compared to the pHF group (P value 0.585; 95% CI -55.53 to 31.62 ; unpaired t -test). The mean breast-feeding period of 114.7 days (SD 82.1; 0.0–270.0 days) did not significantly differ from that in the pHF group (P value 0.742; 95% CI -35.64 to 49.74 ; unpaired t -test), nor did the mean partial formula-feeding period of 143.0 days (SD 81.8; 10.0–268.0 days; P value 0.855; 95% CI -44.82 to 37.28 ; unpaired t -test).

Twelve-month feeding groups. No infants were exclusively breast-fed at the age of 12 months. Thirty-one received pHF and 28 eHF. Two infants (eHF) received only mother's milk with supplementary food but no formula (evening milk pap was made of mother's milk). Three infants (1 pHF, 2 eHF) belonged to the dropout group I, whereas eight (5 pHF, 3 eHF) dropped out to dropout group II (feeding unhydrolyzed formula due to parental noncompliance or poor acceptance of the hydrolyzed formula, or inadvertent feeding of milk proteins in supplementary food).

Twelve-month formula feeding pattern. In the pHF group, 31 infants received pHF with the mean formula-feeding period of 240.2 days (SD 91.0; extreme values 74.0–376.0 days), the mean breast-feeding period of 123.8 days (SD 85.0; 0.0–268.0 days), and the mean partial formula-feeding period of 181.6 days (SD 106.8; 0.0–346.0 days).

In the eHF group, 28 infants received eHF with the mean formula-feeding period of 245.4 days (SD 91.6; extreme values 83.0–392.0 days). No significant difference was detected compared to the pHF group (P value 0.826; 95% CI -42.39 to 52.92 ; unpaired t -test). The mean breast-feeding period of 121.4 days (SD 88.1; 0.0–303.0 days) did not significantly differ from that in the pHF group (P value 0.915; 95% CI -47.57 to 42.75 ; unpaired t -test), nor did the mean partial formula-feeding period of 216.0 days (SD 107.6; 8.0–385.0 days; P value 0.222; 95% CI -21.47 to 90.45 ; unpaired t -test).

Two infants (eHF) received only mother's milk with supplementary food, but no formula (evening milk pap was made of mother milk). Three infants (1 pHF,

2 eHF) belonged to dropout group I, whereas eight (5 pHF, 3 eHF) dropped out to drop-out group II (feeding unhydrolyzed formula due to parental non-compliance or poor acceptance of the hydrolyzed formula, or inadvertent feeding of milk proteins in supplementary food).

Cow's milk-specific PBMC reactivity

The specific reactivity of mononuclear cells of infants from the three feeding groups above at the age of 6 months and the two at the age of 12 months, respectively, was investigated. At 6 months of age, we could test cells from all infants in the particular groups. At 12 months of age, we could test cells from all infants but one (from the pHF group), whose parents refused blood sampling. PBMC from each infant were enough to test with all the antigens given with the exception of one infant from the pHF group at 12 months, whose reactivity was not tested for CAS due to the low cell number. The results of infants from the dropout II group (unhydrolyzed formula) in both ages are not given.

At 6 months of age, a decreased PBMC reactivity to ALA, BLG, CAS, ACAS, and BCAS was found in the pHF-fed infants, as compared with these exclusively breast-fed infants, and between the pHF- and the eHF-fed infants (Table 1). However, the difference proved to be statistically significant only for CAS between pHF and BF, and for ALA, BLG, and ACAS between the pHF and eHF groups, respectively. The PBMC reactivity at 6 months is plotted in Fig. 1 (top).

At 12 months of age, a significantly decreased PBMC reactivity to ACAS was found in pHF-fed infants as compared with the eHF group (Table 1). For example, for BLG reactivity at 12 months, where the difference between pHF and eHF groups was insignificant, the test gave 20% power to detect the smallest interesting difference of 1.0 SI. The PBMC reactivity at 12 months is plotted in Fig. 1 (bottom).

If we set the cutoff value for stimulation index as 2.0 and consider values of ≥ 2.0 as positive, we can use the

Table 1. Reactivity of PBMC to cow's milk antigens at ages of 6 and 12 months, respectively. For 6-month data, Mann-Whitney U-test was used, and for 12-month data unpaired t -test was used

Antigen	Age 6 months						Age of 12 months				
	SI (median)			P values			SI (mean)				P value pHF vs eHF
	pHF $n=24$	BF $n=24$	eHF $n=21$	pHF vs BF	pHF vs eHF	BF vs eHF	pHF $n=29$	pHF (SD)	eHF $n=28$	eHF (SD)	
ALA	1.25	1.64	1.88	0.071	0.016*	>0.1	1.63	1.32	1.73	1.03	>0.1
BLG	1.00	1.56	1.40	0.093	0.042*	>0.1	2.39	1.69	3.29	4.55	>0.1
CAS	1.18	1.70	1.53	0.033*	0.052	>0.1	1.70 ^s	1.20	1.61	0.77	>0.1
ACAS	1.23	1.64	1.97	>0.1	0.020*	>0.1	1.71	0.86	2.26	1.19	0.047*
BCAS	1.16	1.47	1.35	0.065	0.064	>0.1	1.42	0.64	1.57	0.80	>0.1

Statistically significant on 95% significance level.

^s $n=28$ for CAS.

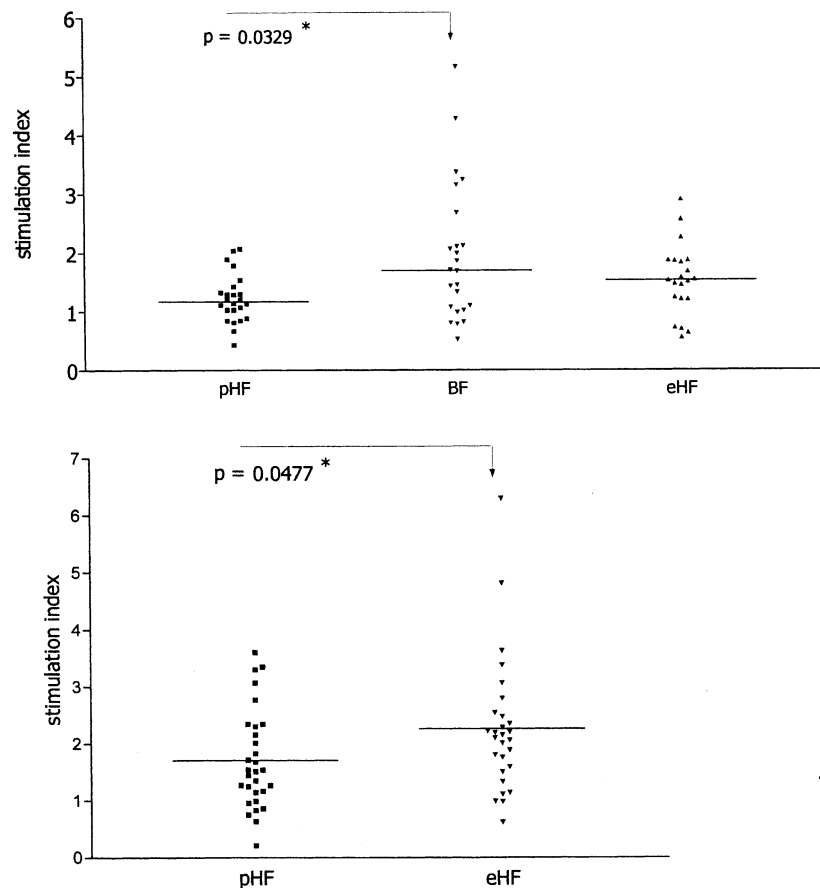


Figure 1. Top: specific reactivity of PBMC to cow's milk casein in infants aged 6 months. Significantly decreased cell reactivity was found in pHF group compared to BF (Mann-Whitney U-test). Horizontal lines indicate medians. Bottom: specific reactivity of PBMC to cow's milk α -casein in infants aged 12 months. Significantly decreased cell reactivity was found in pHF group compared to eHF (unpaired *t*-test). Horizontal lines indicate means.

Fisher's exact test to compare proportions. The results for CAS reactivity at 6 months (number of SI ≥ 2.0 / <2.0) were, for pHF, 2/22, and for BF, 9/15; $P=0.036$. For ACAS at 6 months, the results were as follows: pHF: 3/21; eHF: 9/12, $P=0.041$.

Cow's milk-specific IgG antibodies

Levels of specific IgG antibodies to cow's milk antigens in plasma could be tested at the ages of 6 and 12 months, respectively. At 6 months of age, we could test plasma samples from all infants in the particular groups with the exception of one plasma sample from the pHF group, which was missed. At 12 months of age, we could test cells from all infants but one (from the pHF group), whose parents refused blood sampling. The results of infants from dropout II group (unhydrolyzed formula) at both ages are not given.

An insignificantly higher level of BLG-specific IgG antibody at 6 months was found in the pHF-fed infants than in those who were exclusively breast-fed (25th percentile; median; 75th percentile): pHF: 0.00; 0.08; 0.17; $n=23$; BF: 0.00; 0.00; 0.05; $n=24$; $P=0.137$; Mann-Whitney U-test. Moreover, we found insignif-

icantly higher levels of plasma IgG antibodies to BCAS in infants fed pHF than in those fed eHF at 12 months (25th percentile; median; 75th percentile): pHF: 0.00; 0.14; 0.38; $n=30$; eHF: 0.00; 0.03; 0.14; $n=28$. $P=0.089$, Mann-Whitney U-test. Levels of IgG antibodies to the above antigens are plotted in Fig. 2.

There were nonsignificant differences in the specific IgG levels to other caseins and ALA at both 6 and 12 months. ALA-specific IgG was only rarely detectable.

Cow's milk-specific IgE antibodies

The distributions of positive IgE to cow's milk at 6 and 12 months, respectively, among the feeding groups can be found in Table 2. All infants who developed milk-specific IgE at 6 months had been prenatally randomized to the eHF group. Due to the low numbers of infants, statistical evaluation of the data at both times is not useful.

IgE immunoblotting with plasma samples of cow's milk-specific IgE-positive infants is shown in Fig. 3. Infants with positive milk-specific IgE were sensitized predominantly to α - and β -caseins (25–30 kDa range) followed by β -lactoglobulin (18.8 kDa). The develop-

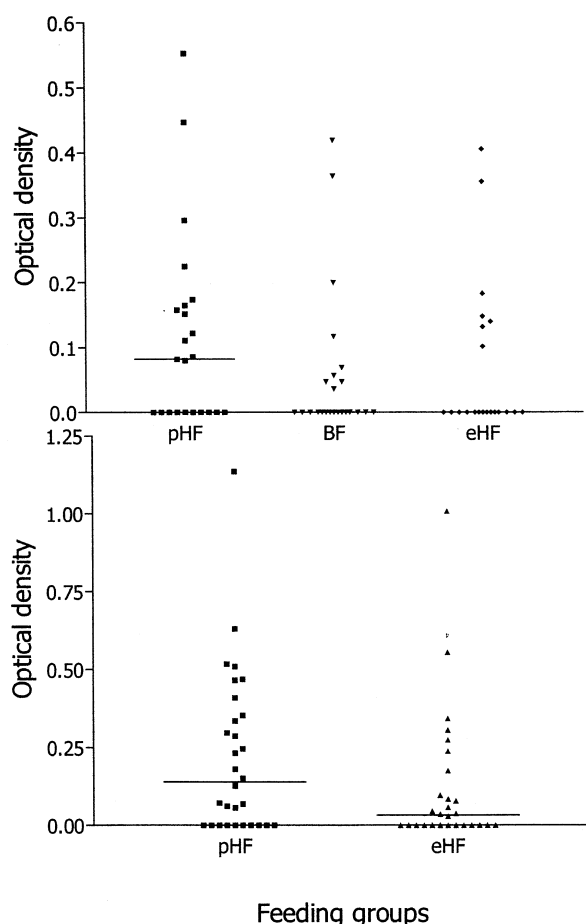


Figure 2. Top: specific IgG antibodies to bovine β -lactoglobulin at 6 months. Horizontal lines indicate medians. Bottom: specific IgG antibodies to bovine β -casein at 12 months. Horizontal lines indicate medians.

ment of IgE levels from 6 to 12 months of age could be followed, and showed no decrease in any infant. Four of these five infants were fed eHF after 6 months of age; patient no. 6 was still breast-fed as his mother refused to feed him eHF.

Additional data of infants whose milk-specific IgE are shown (perinatal formula feeding, duration of exclusive breast-feeding, household smoking, pet keeping, total IgE, etc.) are summarized in Table 3.

Total IgE antibodies

The numbers of plasma samples for the total IgE analysis are identical to those analyzed for specific IgG and IgE.

Plasma concentrations of total IgE did not differ significantly among feeding groups at the respective times of examinations. Results are given (kU/l): (feeding group: examination time; 25th percentile; median; 75th percentile; number of probands). pHF: 6 months; 2.0; 4.0; 27.0; 23. BF: 6 months; 2.0; 4.0; 19.0; 24. eHF: 6 months; 1.0; 2.0; 14.0; 21. pHF vs BF ($P=0.816$); pHF vs eHF ($P=0.590$); BF vs eHF ($P=0.465$); (Mann-

Table 2. Distribution of positive IgE in infants from different feeding groups at 6 and 12 months of age, respectively

Age Feeding groups	6 months			12 months		
	pHF	BF	eHF	pHF	eHF	MM
Positive specific IgE	0	3	2	0	4	1
Negative specific IgE	23	21	19	30	24	1
Total <i>n</i>	23	24	21	30	28	2

MM: infants who received not formula but mother milk and supplementary food.

Whitney U-test). pHF: 12 months; 3.5; 8.0; 18.0; 31. eHF: 12 months; 3.0; 10.0; 27.0; 28. pHF vs eHF $P=0.509$ (Mann-Whitney U-test). Moreover, we used the unpaired *t*-test after logarithmic data transformation; the differences are not significant.

Clinical follow-up

The infants' weights were not significantly different between the respective feeding groups at all times of examinations (data not shown).

Skin symptoms

The numbers of infants with a diagnosis of AD and the use of external corticosteroids are summarized in Table 4. In infants with AD, the mean onset of skin symptoms evaluated at the age of 6 months (in months: mean; SD; *n*) was as follows: pHF: 2.16; 1.10; 7. BF: 1.93; 0.81; 7. eHF: 2.72; 1.62; 6. The mean onset of skin symptoms evaluated at the age of 12 months (months: mean; SD; *n*) was as follows: pHF: 5.28; 2.81; 6. eHF: 3.06; 2.17; 8.

The severity of skin lesions in patients with AD who received no external corticosteroid treatment, shown as the SCORAD points, is listed in Table 5. Due to low infant numbers, no statistical analyses were done.

Discussion

The present study deals with an important issue of the cow's milk immune response and skin symptom development in infants from families with atopy. Most such studies dealt with infants with double-positive family history of atopy and increased cord-blood total IgE antibodies. Our study recruited infants with unfold or multifold history of atopy in first-degree relatives. As we had not confirmed atopy in relatives in our study and simply relied on data from questionnaires and at least-24 months of treatment by allergologists/dermatologists for atopy, the atopic predisposition of infants studied is certainly heterogeneous. Moreover, we had no values of cord-blood total IgE, an additional marker of atopic reactivity development.

However, the present study focused primarily on the cellular cow milk's-specific immune response and on the development of IgG to food antigens, and then on

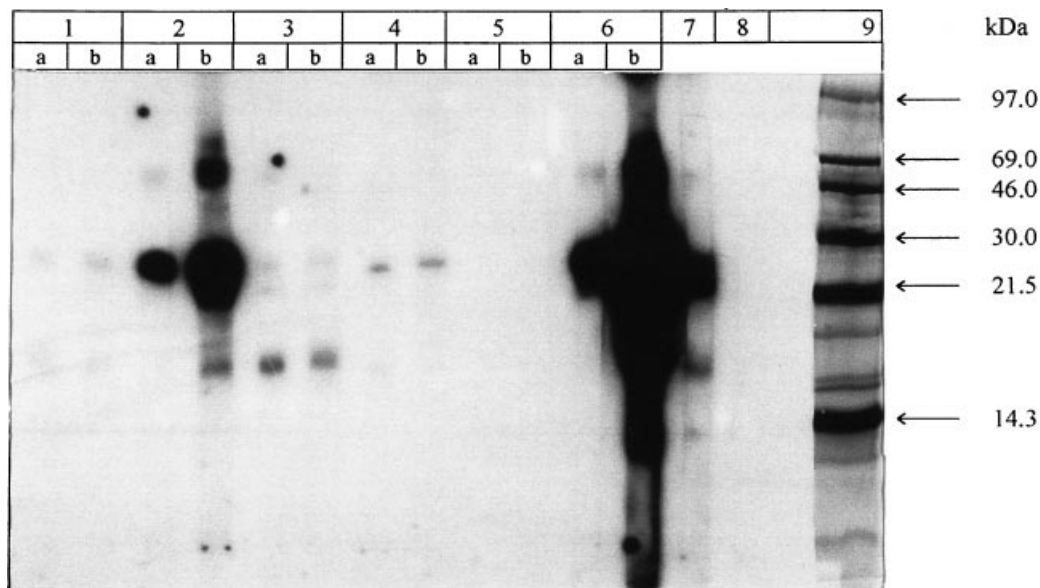


Figure 3. IgE immunoblot of cow's milk proteins with infants' plasma (15% SDS-PAGE, plasma dilution 1:5, I^{125} -autoradiographic detection). Lanes 1 and 4) eHF-fed infants; lanes 2, 3, and 6) exclusively breast-fed infants; lane 5) infant with negative IgE to milk: a) 6-month plasma; b) 12-month plasma lane; lane 7) positive control (2-year-old child with AD; lane 8) negative control (buffer); lane 9) ^{14}C protein molecular weight marker (CFA756, Amersham Pharmacia, Biotec, Uppsala, Sweden).

the cow's milk-specific sensitization. Therefore, we consider adequate the definition of the atopic cohort for this purpose.

The antigen-specific immune response is influenced, firstly, by the genetic predisposition of the reacting organism and, secondly, by the nature of the antigen, its dose, and the duration of exposure, as well as the way antigen is presented to the immune system. Adjuvant factors and maternal immunity are other important influences.

Antigen-specific PBMC reactivity

T cells in peripheral blood recognize an antigen after its presentation by an antigen-presenting cell. It has been revealed that lymphocytes in cord blood are primed with cow's milk antigens and respond to them (18).

However, repeated contact with an antigen can lead to a reduced proliferation in response to this antigen, the state of unresponsiveness.

The finding of a decreased lymphocyte reactivity to particular cow's milk proteins tested in the pHF-fed group can be explained in two ways. Firstly, the administration of highly immunogenic peptides and/or proteins present in pHF results in a loss of lymphocyte reactivity to the respective antigen, a process known as immune deviation that is associated with tolerance induction (20). That would imply that pHF can induce antigen-specific oral tolerance to intact milk antigens. Similar results have been described in a murine model by Fritsché et al. (21), who found an antigen-driven lymphocyte unresponsiveness induced by feeding pHF in contrast to feeding eHF. Secondly, antigen-specific lymphocytes are no longer present in peripheral blood, as they home to the gut. This phenomenon has been

Table 3. Additional data of 6-month-old infants with cow's milk-specific IgE demonstrated in immunoblot. Blot numbers refer to those in Fig. 3

Blot number	Randomization	Perinatal nutrition	Feeding group at 6 months	Age at 6-month visit (days)	Duration of HAF feeding (days)	Human milk feeding (days)	Partial HAF feeding (days)	Positive family history of atopy	Pet keeping	Household smoking	Total IgE at 6 months (kU/l)
1	eHF	eHF	eHF	190	189	1*	37	M, F	No	No	10.0
2	eHF	BF	BF	194	—	194	—	M, F, S	No	No	86.0
3	eHF	BF	BF	176	—	176	—	F	No	No	69.0
4	eHF	BF	eHF	195	39	156*	39	M	No	Father	172.0
5	pHF	BF	BF	161	—	161	—	M	No	No	20.0
6	eHF	BF	BF	194	—	194	—				146.0
S	No	No									

M: mother; F: father; S: sibling.

* Exclusive breast-feeding.

Table 4. Number of infants with AD and external corticosteroid (ECS) treatment from groups with various feeding regimens. Evaluated at 6 and 12 months, respectively

Feeding group	6 months					12 months				
			AD with ECS treatment		Total	Cases of AD		AD with ECS treatment		Total
	Yes	No	Yes	No		Yes	No	Yes	No	
pHF	17	7	5	2	24	25	6	3	3	31
BF	17	7	3	4	24	—	—	—	—	—
eHF	15	6	3	3	21	20	8	3	5	28
MM	—	—	—	—	—	0	2	1	1	2
Dropout II	1	2	1	1	3	3	5	4	1	8
Dropout I	—	—	—	—	Unknown					3

MM: infants fed mother's milk and supplementary food.

described by Miles et al. (22) and Vaarala et al. (23) for infants fed unhydrolyzed cow's milk formula. As the pHF used in our study shows a high degree of residual antigenicity (15) close to unhydrolyzed milk, this explanation of the phenomenon is feasible.

Milk-specific IgG antibodies

The production of specific IgG antibodies to food antigens is a physiologic response when feeding those antigens (24). Our results show that feeding pHF over a longer period (the first 12 months of the child's life) stimulates the milk-specific IgG production more than feeding eHF. This suggests that pHF possesses a stronger immunogenicity than eHF. Stimulation of specific IgG antibody production is much lower during exclusive breast-feeding, as already shown by Tainio et al. (25).

That highly immunogenic protein fragments from partially hydrolyzed infant formula stimulate the production of specific IgG antibodies to cow's milk proteins has been recently shown by Oldæus et al. (26). Our study confirms these findings, although nonsignificant differences were found due to low proband numbers. However, the high levels of cow's milk-specific IgG in some exclusively breast-fed infants (Fig. 2) at 6 months cannot be explained by transplacental IgG passage from mother to fetus, and they remain unclarified. In our opinion, this is an interesting finding showing indirectly the presence of cow's milk casein in mother milk and its immunizing rather than sensitizing effect.

Surprisingly, we found IgG antibodies not only to BLG but also to caseins in pHF, as well as in eHF groups at the age of 6 months. As no supplementary food and no unhydrolyzed milk are fed at this age, this implies occurrence of casein traces in these whey hydrolysates or the presence of protein fragments cross-reacting with anticasein antibodies. Electro-

Table 5. Severity of skin lesions in infants with AD without external corticosteroid treatment (SCORAD points)

	6 months			12 months	
	pHF	BF	eHF	pHF	eHF
<i>n</i>	5	3	3	3	3
Mean	16.02	14.67	22.40	13.30	7.967
Standard deviation	25.13	13.05	8.352	4.763	3.550

phoretic investigations of some hydrolyzed whey formulas confirmed bands at molecular weights close to those of caseins (27).

After supplementary food introduction, unintentional violation of the feeding protocol must be considered, especially when cow's milk may be added in minute amounts to supplementary foods (break, cakes), especially after 9 months of age. In effect, the data of cow's milk-specific IgG at 12 months are difficult to interpret.

Specific IgE sensitization to cow's milk

Out of five infants with positive IgE to milk at the age of 6 months, three were breast-fed, and two were fed eHF. One of them received a partial eHF supplementation; therefore, he was exposed also to food antigens via mother's milk. This finding leads to the already known phenomenon that an exclusively breast-fed infant may be sensitized via mother's milk. The risk of sensitization to food allergens via mother's milk along with development of atopy symptoms can be diminished by an allergen-reduced maternal diet, as previously shown (28), although there is no evidence of its long-lasting effect (5). In our study, some mothers had an unrestricted cow's milk-containing diet.

However, there is a hypothesis that the occurrence of cow's milk antigens in mother's milk is not alone sufficient for sensitization and development of cow's milk-specific IgE in a breast-fed infant. Recent investigations on this topic show no difference in the presence of bovine BLG in the human milk of allergic and nonallergic mothers (29). In addition, cytokines such as TGF- β and interleukin-10, normally present in mother's milk, contribute to the establishment of oral tolerance to food antigens. Their deficiency in mother's milk may lead to cow's milk sensitization in exclusively breast-fed infants, as suggested by Kalliomaki et al. (30). The influence of a maternal history of atopy on this phenomenon is undoubtedly strong although not self-explanatory. In two of three exclusively breast-fed sensitized infants, the mother was allergic.

The most probable explanation for the IgE sensitization of breast-fed babies is a disturbance of human milk immune factors, resulting in the breakdown of oral tolerance to the food antigens normally present in

human milk (31). Further research in human milk immunology and atopy development is needed to test this hypothesis.

Finally, it should be stated that the breast-fed population was not randomized, and therefore the study was burdened with a selection bias. Mothers whose infants develop eczema in the first weeks or months of life tend to breast-feed longer than those whose infants are symptom-free.

Total IgE antibodies

Our data confirm that feeding hypoallergenic formulas cannot influence the overall atopic reactivity, but only modulate the milk-specific response.

Clinical symptoms of atopy

Oldæus et al. (8) found a lower incidence of atopic eczema in infants with a high risk of atopy fed a casein eHF than to infants fed whey pHF, and CMF and those breast-fed, while no difference in IgE sensitization to milk was seen. Similarly, in a large, prospective, double-blind, randomized study, Halken et al. (9) reported the least prevalence of AD in the group fed an extensively hydrolyzed whey formula. We were not able to confirm these findings in our study, but this does not mean there was no difference. The relatively low power of statistical analyses (mostly 10–40%) due to low proband numbers entails the risk of accepting a null hypothesis that is in fact false (an error of the second kind).

Distribution of atopy in relatives was comparable in both randomized groups at the beginning and in the feeding groups at 6 months. In addition to genetic predisposition, there are major confounding variables such as household smoking, pet keeping, low duration of breast-feeding, etc., which we have to control for or at least to consider. Feeding groups in our study were similar in these major confounders. But, mainly, the follow-up in our study was too short to detect true differences.

We could not prove any significant difference in the development of atopic skin symptoms between all groups within the first 12 months of age. As the pathogenesis of atopic disease is very complex, and nutrition is considered to be only a minor factor affecting the development of atopic manifestation, a

long-lasting protection against atopy cannot be expected. The clinical significance of our study is therefore limited, as the data do not allow any recommendation for feeding infants. Large, prospective studies with a profound immunologic background are necessary.

The following conclusions can be drawn from the present study:

- 1) The administration of pHF to infants from families with atopic symptoms induces suppression of their peripheral lymphocytes' specific reactivity to cow's milk caseins at 6 months compared to breast-fed infants, and at 12 months compared to eHF-fed infants.
- 2) Infants fed pHF tend to have higher concentrations of IgG to β -lactoglobulin at 6 and β -casein at 12 months than infants exclusively breast-fed or fed eHF, respectively.
- 3) Our study has confirmed the formerly described phenomenon of specific IgE sensitization to cow's milk proteins in exclusively breast-fed infants.
- 4) Feeding a hypoallergenic formula does not affect the levels of total IgE compared to exclusive breast-feeding.

Even when infants can become sensitized to food allergens by exclusive breast-feeding via mother's milk, there is no justification to change thoughtlessly to a hypoallergenic formula. As before, reliable atopy prevention measures in infants with a high risk of atopy include exclusive breast-feeding for at least 4–6 months, delayed introduction of supplementary food, and avoidance of strong food allergens during at least the first 12 months of age.

Acknowledgments

The study was supported by the Internal Grant Agency of the Health Ministry of the Czech Republic, grants no. 1594-5 and NI-5206/3; by the Bundesministerium für Wissenschaft und Forschung, grant no. 49.847; and by the Fond für wissenschaftliche Förderung, grant no. S6701-MED. The study has been done independently of infant food companies. We thank the neonatologist Dr Černá for help with recruitment of families, the statistician Dr Marie Hladíková for help with the statistical analyses, and the pediatric dermatologist Dr Štěpánka Čapková for consultation.

References

1. WILLEMS R, DUCHATEAU J, MAGREZ P, DENIS R, CASIMIR G. Influence of hypoallergenic milk formula on the incidence of early allergic manifestations in infants predisposed to atopic diseases. *Ann Allergy* 1993;**71**:147–150.
2. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Effect of an allergy prevention programme on incidence of atopic symptoms in infancy. A prospective study of 159 "high-risk" infants. *Allergy* 1992;**47**:545–553.
3. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Preventive effect of feeding high-risk infants a casein hydrolysate formula or an ultrafiltrated whey hydrolysate formula. A prospective, randomized, comparative clinical study. *Pediatr Allergy Immunol* 1993;**4**:173–181.

4. HALKEN S, HØST A. Prevention of allergic disease. Exposure to food allergens and dietetic intervention. *Pediatr Allergy Immunol* 1996;**7**:102–107.
5. ZEIGER RS, HELLER S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J Allergy Clin Immunol* 1995;**1190**.
6. AGGETT PJ, HASCHKE F, HEINE W, et al. Comment on antigen-reduced infant formulae. ESPGAN Committee on Nutrition. *Acta Paediatr* 1993;**82**:314–319.
7. BUSINCO L, DREBORG S, EINARSSON R, et al. Hydrolyzed cow's milk formulae. Allergenecity and use in treatment and prevention. An ESPACI position paper. European Society of Pediatric Allergy and Clinical Immunology. *Pediatr Allergy Immunol* 1993;**4**:101–111.
8. OLDAEUS G, ANJOU K, BJÖRKSTÉN B, MORAN JR, KJELLMAN NI. Extensively and partially hydrolyzed infant formulas for allergy prophylaxis. *Arch Dis Child* 1997;**77**:4–10.
9. HALKEN S, HANSEN KS, JACOBSEN HP, et al. Comparison of a partially hydrolyzed infant formula with two extensively hydrolyzed formulas for allergy prevention: a prospective, randomized study. *Pediatr Allergy Immunol* 2000;**11**:149–161.
10. FALTH-MAGNUSSON K, KJELLMAN NI. Allergy prevention by maternal elimination diet during late pregnancy – a 5-year follow-up of a randomized study. *J Allergy Clin Immunol* 1992;**89**:709–713.
11. HERRMANN ME, DANNEMANN A, GRUTERS A, et al. Prospective study of the atopy preventive effect of maternal avoidance of milk and eggs during pregnancy and lactation. *Eur J Pediatr* 1996;**155**:770–774.
12. ZEIGER RS, HELLER S, MELLON MH, et al. Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: a randomized study. *J Allergy Clin Immunol* 1989;**84**:72–89.
13. ZEIGER RS. Prevention of food allergy in infants and children. *Immunol Allergy Clin North Am* 1999;**19**:619–646.
14. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993;**186**:23–31.
15. SZEPPALUSI Z, NENTWICH I, JOST E, et al. Cord blood mononuclear cells and milk-specific T-cell clones are tools to evaluate the residual immunogenicity of hydrolyzed milk formulas. *J Allergy Clin Immunol* 1998;**101**:514–520.
16. BINDELS JG, BOERMA JA. Hydrolyzed cow's milk formulae. *Pediatr Allergy Immunol* 1994;**5**:189–190.
17. KONDO N, KOBAYASHI Y, SHINODA S, et al. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992;**67**:1003–1007.
18. SZEPPALUSI Z, NENTWICH I, GERSTMAYR M, et al. Prenatal allergen contact with milk proteins. *Clin Exp Allergy* 1997;**27**:28–35.
19. SZEPPALUSI Z, EBNER C, URBANEK R, et al. Detection of IgE antibodies specific for allergens in cow's milk and cow dander. *Int Arch Allergy Immunol* 1993;**102**:288–294.
20. STROBEL S. Dietary manipulation and induction of tolerance. *J Pediatr* 1992;**121**:S74–S79.
21. FRITSCHÉ R, PAHUD JJ, PECQUET S, PFEIFER A. Induction of systemic immunologic tolerance to beta-lactoglobulin by oral administration of a whey protein hydrolysate. *J Allergy Clin Immunol* 1997;**100**:266–273.
22. MILES EA, WARNER JA, JONES AC, COLWELL BM, BRYANT TN, WARNER JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996;**26**:780–788.
23. VAARALA O, SAUKKONEN T, SAVILAHTI E, KLEMOLA T, AKERBLUM HK. Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *J Allergy Clin Immunol* 1995;**96**:917–923.
24. FALTH-MAGNUSSON K, KJELLMAN NI, MAGNUSSON KE. Antibodies IgG, IgA, and IgM to food antigens during the first 18 months of life in relation to feeding and development of atopic disease. *J Allergy Clin Immunol* 1988;**81**:743–749.
25. TAINIO VM, SAVILAHTI E, ARJOMAA P, SALMENPERA L, PERHEENTUPA J, SIIMES MA. Plasma antibodies to cow's milk are increased by early weaning and consumption of unmodified milk, but production of plasma IgA and IgM cow's milk antibodies is stimulated even during exclusive breast-feeding. *Acta Paediatr Scand* 1988;**77**:807–811.
26. OLDAEUS G, BJÖRKSTÉN B, JENMALM MC, KJELLMAN NI. Cow's milk IgE and IgG antibody responses to cow's milk formulas. *Allergy* 1999;**55**:352–357.
27. RESTANI P, VELONA T, PLEBANI A, et al. Evaluation by SDS-PAGE and immunoblotting of residual antigenicity in hydrolyzed protein formulas. *Clin Exp Allergy* 1995;**25**:651–658.
28. SIGURS N, HATTEVIG G, KJELLMAN B. Maternal avoidance of eggs, cow's milk, and fish during lactation: effect on allergic manifestations, skin-prick tests, and specific IgE antibodies in children at age 4 years. *Pediatrics* 1992;**89**:735–739.
29. HØST A, HUSBY S, HANSEN LG, ØSTERBALLE O. Bovine beta-lactoglobulin in human milk from atopic and non-atopic mothers. Relationship to maternal intake of homogenized and unhomogenized milk. *Clin Exp Allergy* 1990;**20**:383–387.
30. KALLIOMAKI M, OUWEHAND A, ARVILOMMI H, KERO P, ISOLAURI E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 1999;**104**:1251–1257.
31. JÄRVINEN KM, JUNTUNEN BK, SUOMALAINEN H. Relation between weak HLA-DR expression on human breast milk macrophages and cow's milk allergy (CMA) in suckling infants. *Pediatr Res* 1999;**45**:76–81.