

Cow's milk protein allergy and intolerance in infancy

Some clinical, epidemiological and immunological aspects

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Reproducible clinically abnormal reactions to cow's milk protein (CMP) may be due to the interaction between one or more milk proteins and one or more immune mechanisms, possibly any of the four basic types of hypersensitivity reactions. At present, evidence for type I, III and IV reactions against CMP has been demonstrated. Immunologically mediated reactions, mainly immediate IgE-mediated reactions are defined as cow's milk protein allergy (CMPA). Non immunologically reactions against CMP are defined as cow's milk protein intolerance (CMPI). Many studies on "cow's milk allergy" have not investigated the immunological basis of the clinical reactions. It is not possible to differentiate between CMPA and CMPI solely on clinical symptoms. No single laboratory test is diagnostic of CMPA/CMPI. Therefore, the diagnosis still has to be based on strict well-defined elimination and milk challenge procedures. Before 1950 CMPA/CMPI was rarely diagnosed. Since 1970 widely varying estimates of the incidence from 1.8% to 7.5% have been reported, mainly reflecting differences in diagnostic criteria and study design. Based on strict diagnostic criteria the incidence of confirmed CMPA/CMPI in infancy seems to be about 2–5% in developed countries. Symptoms suggestive of CMPA/CMPI may be encountered in about 5–15% of infants emphasizing the importance of controlled elimination/milk challenge. In breastfed infants reproducible clinical reactions to CMP in human milk have been reported in about 0.5%. Most infants with CMPA/CMPI develop symptoms before one month of age, often within one week after introduction of cow's milk based formula. The majority have ≥ 2 symptoms and symptoms from ≥ 2 organ systems. About 50%–70% have cutaneous symptoms, 50–60% gastrointestinal symptoms, and about 20–30% respiratory symptoms. In exclusively breast-fed infants with CMPA/CMPI severe atopic eczema is a predominant symptom. Debut of CMPA/CMPI after 12 months is extremely rare. The basic treatment is complete avoidance of CMP. In infancy a proven hypoallergenic CM substitute is needed. Due to clinically important residual allergenicity in some hypoallergenic formulae controlled clinical testing is necessary in each case before use. Goat's milk proteins share identity with CMP. Raw untreated cow's milk and unhomogenized cow's milk is as allergenic as normal pasteurized and homogenized milk products. The prognosis of CMPA/CMPI is good with a remission rate about 45–50% at one year, 60–75% at two years, and 85–90% at three years. Associated adverse reactions to other foods develop in about 50%, and allergy against inhalants in 50–80% before puberty. High levels of IgE to CMP are associated with an increased risk of persisting CMPA, persisting allergy to other foods and inhalant allergy. The degree of antibody response to CMP exposure is a better predictor of persisting CMPA than a single finding of an elevated antibody level which can occur in normal infants without CMPA/CMPI. Cow's milk proteins are absorbed from the gut and can be measured in the blood in

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quantities of micrograms per liter serum both in children and adults. Macromolecular absorption is increased in preterm infants and in newborns, and has also been demonstrated in infants with CMPA. Whether an increased macromolecular absorption is a part of an allergic constitution/heredity or is due to temporal mucosal damage is not clarified, and the relevance of an increased absorption for development of clinical disease is unclear. The cow's milk protein, betalactoglobulin can be detected in the breast milk of 95% of lactating women (0.9–150 µg/l, median value 4.2 µg/l) when consecutive continual testing of breast milk is done. Considering the low frequency of CMPA/CMPI these small amounts of CMP in breast milk may rather induce tolerance to CMP than allergic sensitization. The development of CMPA/CMPI primarily seems to depend on the exquisite immune responsiveness in predisposed individuals. A significant association between early neonatal exposure to cow's milk formula feeding and subsequent development of CMPA/CMPI has been documented. Preliminary findings of specific IgE to individual cow's milk proteins in cord blood of the majority of infants with later development of CMPA/CMPI may indicate intrauterine sensitization playing a role in the pathogenesis of CMP/CMPI, perhaps as a weak intrauterine priming inducing a low IgE response, which needs to be boosted postnatally in order to cause clinical disease, especially in infants with a marked atopic predisposition.

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Definitions

Adverse reaction

Reproducible clinically abnormal response to a substance which usually does not give any reactions.

Allergenicity

The ability of a protein (allergen) to induce allergic reactions.

Allergic rhinitis/conjunctivitis

Rhinitis or conjunctivitis appearing at least twice caused by exposure to a particular allergen, and eosinophilia in nasal smear at symptoms (above 10% eosinophils).

Allergic urticaria

Urticaria appearing at least twice caused by exposure to a particular allergen.

Allergy

The term "allergy" was originally introduced by von Pirquet 1906 (1) to indicate a changed reaction to foreign substances implying evidence of clinical hypersensitivity. Since the discovery of IgE in 1967 (2) many have equated allergy with IgE-mediated responses, because the majority of allergic reactions seems to be IgE-mediated. As a variety of immunological mechanisms may be involved in allergic reactions a more comprehensive definition including any of the four basic types (I-IV) of hypersensitivity

reactions classified by Gell & Coombs (3) is preferable.

Atopic dermatitis, atopic eczema

Pruritic, chronic or chronically relapsing non-infectious dermatitis with typical morphology and distribution as suggested by Hanifin & Rajka, 1980 (4).

Atopic manifestations

Symptoms of atopic dermatitis, bronchial asthma, allergic rhinitis/conjunctivitis, allergic urticaria and/or gastrointestinal allergy.

Cow's milk protein allergy

Proven or likely immunologically mediated (any of types I-IV) reactions to cow's milk protein associated with reproducible adverse reactions, giving symptoms from the gastrointestinal tract, the skin, or the respiratory tract.

Cow's milk protein intolerance

Reproducible adverse reactions to cow's milk protein giving symptoms from the gastrointestinal tract, the skin or the respiratory tract. Immunological etiology not confirmed, either due to lack of immunological investigations or negative results of the undertaken analyses.

Diarrhoea

Change of bowel movements (> 3/day) and loose stools.

Food allergy

Proven or likely immunologically mediated reactions to food(s) associated with reproducible adverse reactions, giving symptoms from the gastrointestinal tract, the skin, or the respiratory tract.

Food intolerance

Reproducible adverse reactions to food(s) or food additives giving symptoms from the gastrointestinal tract, the skin or the respiratory tract; immunological etiology not demonstrated.

Immunogenicity

The ability to induce an immune response.

Infantile colic

Intermittent excessive crying (> 2 h/day) for at least one week without any obvious cause.

Recurrent wheezing

Two or more episodes of expiratory wheezing with or without concomitant upper airway infection.

Sensitization

The first induction of an allergic immune response at exposure to a particular allergen.

Abbreviations

AB	Antibody
AG	Antigen
ALA	Bovine alphasalactalbumin
BIG	Bovine immunoglobulin
BLF	Bovine lactoferrin
BLG	Bovine betalactoglobulin
BSA	Bovine serum albumin
CAS	Bovine casein
CB	Cord blood
CB-IgE	Cord blood IgE
CM	Cow's milk
CMP	Cow's milk protein
CMPA	Cow's milk protein allergy
CMPI	Cow's milk protein intolerance
CRIE	Crossed radioimmuno-electrophoresis
DBPCFC	Double-blind placebo controlled food challenge
ELISA	Enzyme-linked immunosorbent assay
HPLC	High performance liquid gel permeation chromatography
IgG	Immunoglobulin class G
IgM	Immunoglobulin class M
IgA	Immunoglobulin class A
IgE	Immunoglobulin class E
OA	Ovalbumin

RAST	Radioallergosorbent test
SPT	Skin prick test

Introduction and historical background

Adverse reactions to CM were first described for more than 2,360 years by Hippocrates (prior to 370 BC), who is said to have observed that CM could cause gastric upset and urticaria (5). Five hundred years later Galen described a case of milk allergy (6). In the first years of this century reports on adverse reactions to CM appeared in the German literature describing symptoms such as diarrhoea and failure to thrive (7, 8). Finkelstein (9) published the first case of anaphylactic shock and death caused by CM ingestion. In 1906 Moro (10) demonstrated precipitins to CM in blood from a marasmic infant fed CM. In Scandinavia Wernstedt (1910) was the first to describe a case of "idiosyncrasy" to cow's milk in Sweden (11), and he proposed the term allergy to CM supposing a changed reactivity to CM because of previous exposure to CM. In Denmark Hertz (1914) reported the first case of "CM idiosyncrasy" (12). Halberstadt (13) showed that the whey fraction of CM caused adverse intestinal symptoms. The American pediatrician Talbot (1916) clarified the relation between "idiosyncrasy to CM" and anaphylaxis to CM in two infants (14). Later, urticaria (15) was described as an adverse reaction to CM. In the following decades CMPA/CMPI was considered an infrequent disease, and only a few cases were described as reviewed by Vendel (Stockholm, Sweden), who reported the incidence of CM idiosyncrasy in the period 1919–1947 to be about 0.1 o/oo – 0.3 o/oo (16). In the same period a few Danish casuistic reports were published (17–19). Since the 1950s several reports on CMPA/CMPI have shown an increasing frequency of this disease (20), possibly in part caused by a simultaneous decrease in breast-feeding (21) due to more frequent use of cow's milk based infant formula feeding.

Most infants tolerate CM formula without untoward effects. However, CMPA/CMPI has become a common disease in early childhood, and among parents increased attention has been paid to this disease during the last decades. Many reviews (20, 22–30) on CMPA/CMPI have elucidated mainly epidemiological, clinical and diagnostic aspects, but still much controversy exists probably because of a lack of standard terminology and reliable diagnostic tests.

Definition and diagnostic criteria of CMPA/CMPI**Definition**

The subject of cow's milk allergy (and food allergy in general) has suffered from confusion due to lack of a standard terminology. Adverse reactions to milk

(and other foods), which are not immunologically mediated, may be caused by protein intolerance, pharmacological reactions, absorptive defects, enzyme deficiencies (disaccharidases), toxic or infectious reactions and psychological mechanisms. At suspected adverse reactions to CM these possibilities should be considered before diagnostic procedures are performed to prove immunological mechanisms of CMP hypersensitivity. Clinical and laboratory observations indicate that clinical hypersensitivity to CMP may be evoked by heterogeneous immunological mechanisms, in fact any of the four basic types of hypersensitivity reactions classified by Gell & Coombs (3) may be involved, and more than one type of reaction may be seen in a particular patient, even when there is a single clinical manifestation (31). So, in CMPA (and food allergy and intolerance in general) no reliable single diagnostic test is available. In most instances of CMP hypersensitivity only diagnostic studies (such as SPT and RAST) indicative of type I reactions (IgE-mediated) are carried out allowing only to discern IgE-mediated CMPA. Strictly speaking, CMPA cannot be ruled out unless extensive diagnostic tests for type II-III-IV reactions have turned out negative. However, at present this cannot be done by any single or a practical combination of tests. Furthermore, in many instances the immunological mechanism causing the disorder cannot be defined by current techniques. Therefore, the diagnosis of CMPA/CMPI has to be based on the clinical response to CMP.

Diagnostic criteria

In 1963 Goldman et al. (32) proposed strict clinical criteria for the diagnosis of CMPA/CMPI ("milk allergy" in common usage). Goldman's criteria, the accepted mode of diagnosis for 10–15 years, were the following:

1. Symptoms must subside after milk elimination.
2. Symptoms must occur within 48 h following a trial feeding of milk.
3. Three such challenges must be positive and have similar onset, duration and clinical features.
4. Symptoms must subside after each challenge reaction.

As challenge dose 100 ml of skim milk (containing 3.5 g of milk protein) was used. The patients were observed 3–4 h after challenge. However, using these criteria lactose intolerance would be included, and patients with delayed reactions (33) would be excluded wrongly. Furthermore, challenge doses and schedules should be standardized as well as the duration of the milk elimination period should be

defined. In addition a challenge dose of 100 ml of skim milk given at one time may be hazardous, and in fact Goldman (32) reported anaphylaxis in 8/89 patients (9%). It also appears unacceptable to challenge three times at short intervals. Other investigators (34) would accept one or two positive challenges, and in most cases it is sufficient to observe subsidence of symptoms after elimination of CM, recurrence of symptoms during one challenge test and subsidence of symptoms after renewed elimination of CM (20, 26, 35). Some authors (25, 26) claim that double-blind, placebo-controlled, food challenge (DBPCFC) is the only way (gold standard) to confirm the diagnosis of food allergy in order to exclude bias of patient, mother, or physician. However, as pointed out by Hill (37) DBPCFC is not practicable in patients with delayed reactions, because prolonged double-blind, controlled administration of CPM or placebo in high doses (corresponding to normal daily intake of the food) for possibly several days will be difficult to accomplish. In patients with immediate reactions DBPCFC is feasible and recommendable. However, if DBPCFC is negative, an open controlled challenge including normal daily intake of the food protein in question should be carried out to rule out the occurrence of delayed reactions.

Other disorders causing recurrent symptoms on CM challenge should be excluded, especially lactose intolerance and intestinal infection (or sequelae from infection). Lactose intolerance (due to lactase deficiency) may be primary or secondary. The primary forms consist of the excessively rare congenital form (infant diarrhoea on breast milk due to a high lactose content), and the vastly more common late onset or racial form occurring between the age of 4 and 18 years. The secondary lactose intolerance may occur as a transient lactase deficiency due to a variety of diseases with damage of the mucosa of the small intestine (malabsorption, infections etc.) (28). Lactose intolerance can be excluded in babies tolerating human milk, and later by a normal lactose tolerance test (blood glucose rise of at least 1.1 mmol/l, and no symptoms following intake of lactose 2 g/kg body-weight (38, 39).

In most recent investigations (40–46) the diagnosis of CMPA/CMPI was established according to the criteria proposed by Savilahti (20) and Bahna & Gandhi (26):

- 1) definite disappearance of symptoms after each of two dietary eliminations of CM and CM products,
- 2) recurrence of identical symptoms after one challenge, and
- 3) exclusion of lactose intolerance and coincidental infection.

Table 1. Incidence of CMPA/CMPI

Study	Year	Population	Country	Number with CMPA/CMPI	Incidence %
<i>Retrospective</i>					
Vendel (16)	1948	–	Sweden	1/7500	0.01
Stintzing & Zetterström (47)	1979	4311 infants	Sweden	25 admitted to the hospital, severe cases?	0.58
<i>Prospective</i>					
Collins-Williams (48)	1956	3000 a	USA	89?	0.3
Bachman & Dees (49)	1957	304 b	USA	4	1.3
Johnstone & Dutton (50)	1966	240 b	USA	2	0.8
Halpern et al. (51)	1973	1084 b c	USA	20	1.8
		669 b d		6	0.9
Gerrard et al. (52)	1973	787 b	Canada	59	7.5
Jakobsson & Lindberg (39)	1979	1079 b	Sweden	20	1.9
Hide & Guyer (57)	1983	609 b e	England	15	2.5
Bock (53)	1987	480 b	USA	25 f	5.2 f
				11 g	2.2 g
Høst et al. (40)	1988	1749 b	Denmark	39	2.2
		One year birth cohort			
Schrander et al. (58)	1993	1158 h	Holland	26	2.3 i

a Patients followed in a paediatric practise for non allergic reasons. Prospective?

b Healthy babies, unselected.

c CM in the diet from birth

d No CM before 6 months

e 609 infants of a 1-year cohort of 904 newborns followed up to 2 years of age.

f Diagnosed by open challenge (14) and DBPCFC (11)

g Diagnosed by DBPCFC

h 1158 of 1386 unselected infants born between 1985 and 1988 and prospectively followed in 5 health care centres up to 1 yr.

i Diagnosed by two positive open challenges. Using Kaplan-Meier estimate the figure was 2.8% (confidence interval 1.8%–3.8%).

Provided that these criteria are fulfilled, and milk challenge is performed under controlled close professional observation in hospital or at a clinic, over-diagnosis of CMPA in infancy does not seem to be frequent, nor when open milk challenges are used (39, 44). Thus, in the latter study (44) the results of the first 10 positive open milk challenges in infants below 1 year of age were confirmed by DBPCFC as described in (41), and consequently the following milk challenges were carried out in an open controlled design.

In solely breastfed infants, elimination and challenge procedures should be performed via regulation of the mothers' intake of cow's milk.

In any case a well defined standardized challenge schedule should be followed, including description of time intervals between increasing amounts of CMP and total amount of CMP before reaction (35, 39, 44). In research the diagnosis of CMPA/CMPI should be confirmed by DBPCFC.

Incidence

Total incidence

Estimates of the incidence of CMPA/CMPI vary from 0.1% to 7.5% (16, 39, 40, 46–53, 57, 58) (Table 1). This variation mainly reflects differences in criteria for diagnosis, differences in study design (retrospective versus prospective), different composition of the materials, and maybe also geographical differences.

Apparently CMPA/CMPI was a rare disease before 1950–1960. If the reported increase of the incidence of the disease after 1960 is true, one might suppose a relation between this increase and the enormously increased exposure to cow's milk-based infant formulas and the corresponding decrease in breast-feeding during that period in developed countries (21). In Denmark the average duration of breast-feeding dropped from about 24 weeks in 1950 to a minimum of 10 weeks in 1970 (54). Nowadays, breast-feeding is increasing in most developed countries. In Finland an increase in average duration of breast-feeding from 1.7 months in 1971 to 5.7 months in 1983 has been reported (55). Maybe, a decreasing incidence of CMPA/CMPI is to be ex-

pected? Thus, a decreasing frequency of CMPI in Spain has been supposed to reflect a declining use of CM formulae with a high CMP content (56).

In our Danish investigation a cohort of 1749 newborns was followed prospectively for the development of CMPA/CMPI during the first year of life (40, 44). A total of 117 infants (6.7%) had symptoms suggestive of CMPA/CMPI, and based on well-defined elimination/open milk challenge procedures (40, 44) a total of 39 (2.2%) (20 girls and 19 boys) were diagnosed as having CMPA/CMPI. In 78 infants the suspicion could be disproven, and they all still tolerated cow's milk at follow-up at 12 months. None developed CMPA/CMPI after 12 months of age (44). Thus, the total incidence of CMPA/CMPI during the first year of life was 2.2% in unselected Danish infants. This incidence is comparable with the corresponding figures of recent prospective studies by Jakobsson & Lindberg from Sweden (39), and Bock from USA (53), reporting incidences of 1.9% and 2.2%–5.2%, respectively. In the latter study the incidence of 2.2% was confirmed by blind milk challenge, and a further 3.0% by open milk challenge. In the former study the design and open challenge procedure was comparable with that of our study. Similar incidences of CMPA/CMPI were reported from an English (2.5%) and a recent Dutch study (2.8%) applying partly comparable prospective study designs (57, 58). A higher prevalence of 7.5% reported by Gerrard et al. (52) may be explained by different diagnostic criteria mainly based on patient history and challenges carried out at home.

In prospective longitudinal studies of unselected newborns the primary materials of infants with symptoms suggestive of CMPA/CMPI have varied between 4.8% (39), 6.7% (44) and about 15% (53), probably reflecting different study designs, especially regarding inclusion criteria and the number of and intervals between clinical examinations. Interestingly, in infants suspected of CMPA/CMPI the diagnosis was only proven in between 1/3 (44, 53, 59) and 40% (39) when controlled elimination/challenge was used. These studies confirm the importance of avoidance of diagnosing CMPA/CMPI mainly on history of symptoms suggestive of CMPA/CMPI. Open milk challenges were used, and following the described approach (39, 40, 44) overdiagnosis does not seem to be a serious problem in infants, although it would have been optimal to have performed a DBPCFC in all patients (36). In contrast, a milk elimination period of > 4 weeks (40, 44) may have led to a slight underestimation of the "true incidence" of CMPA/CMPI because of the possibility of a resolution of the disease in some patients in the elimination period before challenge.

Optimally, all infants of a cohort should be investigated at short intervals by the same physician (or a few physicians) in order to register all possible symptoms related to intake of CM, and in case of reproducible or persisting symptoms after intake of CM products controlled elimination/challenge procedures should be carried out. In this way, the influence of different thresholds of parents' perception of possible symptoms of disease (CMPA/CMPI) might be at least in part eliminated. However, such a close follow-up of large cohorts of newborns is very time and resource consuming and hardly practicable. The influence of differences in study design in the same study population is shown in one study (39), in which a group of infants followed closely revealed an incidence of CMPA/CMPI of 3.7% versus 1.1% among infants followed up within the ordinary health system. Such differences should be minimized by thorough information of and collaboration with relevant health personnel and parents about study design, inclusion criteria etc. before and during the study as described in (39) and (40, 44).

In conclusion, at present the "true incidence" of CMPA/CMPI in infancy seems to be about 2%–5% in developed countries. Symptoms suggestive of CMPA/CMPI are seen in about 5%–15% of infants, but in about 2/3 of these instances a cause effect relationship between CM and symptoms can be disproven by controlled elimination/milk challenge procedures.

Incidence in exclusively breast-fed infants

In a Danish study (40, 46) the 1-year incidence of CMPA/CMPI in exclusively breast-fed was found to be 0.5% (9/1749) in a study population with a frequency of solely breast-feeding of 52% at 3 months of age (24). A comparable incidence of about 0.4% could be calculated from the data reported in a Swedish study (39). Both studies confirm the old findings by O'Keefe (1920) that breast-fed infants may react to cow's milk ingested by the mother and transferred to the infant via the breast-milk (60). Whether these findings support the hypothesis that cow's milk protein sensitization can occur *in utero*, or via breast-milk or none of these, will be discussed later.

Clinical features

Onset of symptoms

Symptoms of CMPA/CMPI may appear during the period of breast-feeding (39, 40, 52, 60, 61), but mostly start during bottle feeding. As reported in 7–15% of cases (39, 62, 63) symptoms may occur at the first cow's milk containing meal. In contrast, (40) none of 39 infants with CMPA/CMPI had

Table 2. Presenting symptoms (%) in CMPA/CMPI

Symptoms	Selected patient samples			Unselected prospectively followed patients from birth cohorts		
	Goldman et al. (32) 1963 a n 89	Gerrard et al. (64) 1967 b n 150	Hill et al. (67) 1986 c n 100	Gerrard et al. (52) 1973 d n 59	Jakobsson & Lindberg (39) 1979 e n 20	Høst & Halcken (44) 1990 f n 39
Atopic eczema	35	13	13	46	50	56
Colic	28	19	14	20	35	46
Vomiting	33	34	41	22	50	38
Diarrhoea	37	47	48	41	25	8
Recurrent wheezing	27	17	29	12	–	28
Rhinitis	35	29	21	31	–	21
Urticaria	11	–	10	–	20	15
Failure to thrive	–	–	22	–	10	8
Other / e.g. CNS	18	–	–	–	–	0
Diarrhoea with blood	–	13	4	–	–	0
Bronchitis	–	53	–	17	10	–
Exanthema	–	–	6	–	35	–
Anaphylaxis	9	–	2	–	–	0
Angio-oedema	–	–	13	–	–	–
Circumoral lesions	–	–	26	–	–	–
Irritability	–	–	40	–	–	–
Syncope	–	–	12	–	–	0
Convulsion	–	–	2	–	–	0
Anemia	–	–	2	–	–	–
Gastroesophageal reflux	–	–	6	–	–	–
Monosymptomatology	23	–	–	–	25	8

– Figures not given

a Age at investigation: Group A median 6 months (2 wk – 6 yr)
Group B median 10 months (6 wk – 13 yr)

b Age at investigation: not given

c Age at investigation: mean 16 months (3 – 66 months)

d Age at investigation: not given, but infants followed 0 – 2 yr

e Age at investigation: median 4 months (3 wk – 1 yr)

f Age at investigation: median 3.5 months (1–11 months), infants followed 0–3-yr

symptoms at the first intake of CM formula. The age at which symptoms start depends on the time of introduction of CM based formula. Most infants with CMPA/CMPI develop symptoms shortly after introduction of CM formula, within one week in 41% (52), 49% (40), 56% (64) and 70% (39), and within 1 month in 69%, 69%, 75%, and 85% of the infants in the above mentioned studies.

Regarding chronological age Goldman et al. (32) reported onset of symptoms before 1 month in 58%, and before 3 months in 90%. In recent studies (39, 47) the corresponding figures were about 30% and 60%, respectively, the latter values probably reflecting a longer duration of breast-feeding. Bock (53) reported debut of symptoms before 12 months in 24/25 (96%) of infants with CMPA/CMPI followed until 3 years. Likewise, Zeiger et al. (65) and Høst & Halcken (44) found that none of prospectively followed infants developed CMPA/CMPI after 12 months of age.

Goldman et al. (32) reported different ages of onset for different symptoms. Thus, atopic eczema usually occurred after 1 month, vomiting after 1–2

months, colic after 2–3 months and diarrhoea after 3–6 months. Concerning other symptoms no typical age of debut was seen. Similar findings have not been reported by other investigators.

In conclusion – Many studies confirm that CMPA/CMPI develops in early infancy, rarely after 12 months of age. The onset of disease is in most cases closely related to the time of introduction of cow's milk based formula.

Symptomatology

A broad spectrum of symptoms and signs in children may be attributed to ingestion of CM. Mostly reactions are localized to the gastrointestinal tract (“gastrointestinal allergy”), but CMPA/CMPI may also cause systemic symptoms such as anaphylaxis and local reactions in the skin and the respiratory tract. The symptoms may occur within a few minutes – 1 h after milk exposure (i.e. “immediate reactions”), or after 1 h (“delayed reactions”), and in some cases after days.

The classical gastrointestinal symptoms are vomiting, diarrhoea, and colic. Dermatologic symptoms are atopic dermatitis, urticaria, angioedema and contact dermatitis. Respiratory features are recurrent wheezing, stridor, cough and rhino-conjunctivitis. Anaphylaxis has been reported in 9% and 5% (32, 66). Table 2 shows the frequencies of the most common symptoms in various studies (32, 39, 44, 52, 64, 67). Similar symptoms and frequencies were recently reported by Ventura et al. (66) from an Italian multicenter study on CMPA/CMPI in the first year of life (125 cases). Irritability and colic (67) appear to be common symptoms, but nearly always in combination with other symptoms (32, 67). There is some evidence that colic may be caused by CMP in solely breast-fed infants (68,69) as well as by CM formula (70, 71). Sleeping disorders in infants have been related to CMPA/CMPI (72–74), but further studies by other groups seem required to establish a cause-effect relationship between cow's milk and sleeping disorder as the only sign of CMPA/CMPI. However, secondary sleeping disorders due to e.g. itching atopic dermatitis or other atopic manifestations provoked by CMPA/CMPI are clinically well-known. Recurrent otitis has also been reported (32) in a few cases, apparently secondary to allergic rhinitis.

Several well-defined rare diseases with a delayed onset pattern due to CMPA/CMPI have been identified: milk induced colitis (75, 76), cow's milk enteropathy (77–80), eosinophilic gastroenteritis (81, 82), milk induced pulmonary disease, pulmonary hemosiderosis or Heiner's syndrome (83), iron-losing enteropathy and anemia (84), neonatal thrombocytopenia – TAR (85). Other disorders which have been reported in the literature to have a delayed onset delayed onset relationship to milk ingestion are migraine (86), a lupus erythematosus-like syndrome and nephrosis mentioned by Heiner & Wilson (87).

Table 2 (32, 39, 44, 52, 64, 67) shows some differences in the occurrences of common symptoms of CMPA/CMPI. These differences may be ascribed to especially dissimilarities regarding study designs, diagnostic criteria, composition of the patient materials, e.g. different age distributions of the patients. Thus, some authors report the symptomatology of selected groups of patients with CMPA/CMPI (32, 64, 67), while others describe unselected prospectively followed infants with CMPA/CMPI from birth cohorts (39, 44, 52).

It is noteworthy that the vast majority (75–92%) of infants with CMPA/CMPI has more than one symptom (32, 39, 44). In the latter study (44) the three monosymptomatic patients had severe atopic eczema. In the same study, the distribution of cutaneous (64%), gastrointestinal (59%) and respiratory

symptoms (33%) was similar to that of previous prospective population studies (39, 52), whereas diarrhoea occurred in only 8% compared to 25%–41% in the other studies (39, 52). An infrequent occurrence of diarrhoea (44) may be due to a nearly total exclusion of secondary deficiency of lactase on account of a long milk elimination period (> 4 weeks), exclusion of gastrointestinal infections, and perhaps a strict definition of diarrhoea may contribute to this difference. It should be borne in mind that confounding factors such as outdoor and indoor pollution, e.g. exposure to tobacco smoking, may act as an adjuvant factor and influence the symptomatology of patients with CMPA/CMPI, especially regarding respiratory symptoms. Thus, 56% of the newborns followed prospectively regarding development of CMPA/CMPI (40, 44) were daily exposed to passive smoking at home (88), and 10/11 infants with recurrent wheezing at cow's milk exposure (44) were daily exposed to passive smoking.

Symptomatology in exclusively breast-fed infants

In exclusively breast-fed infants with CMPA/CMPI reactions against CMP in the mother's milk have been registered > 4 h after the mother's intake of cow's milk (40). The symptomatology is similar to that encountered in other infants with CMPA/CMPI (40, 44, 61, 89), although severe atopic eczema seems to be a more predominant symptom in that subgroup (40, 61, 89).

Clinical and immunological types of reaction

Previous reports have in detail discerned two main groups according to the time of onset of reaction after milk challenge *a) immediate onset*, within 1 h and *b) delayed onset* after 1 h after milk challenge (35, 90). More than 40 years ago the same observations were mentioned by Vendel (16). According to Ford et al. (35) children with immediate onset reactions usually had cutaneous manifestations, positive SPT, raised IgE values, were atopic, and the reaction was provoked by only small amounts of milk. Children with delayed onset reactions usually had gastrointestinal manifestations, negative SPT, normal IgE values, were non atopic, and had a history of vomiting, diarrhoea and colic in the first year of life, and larger amounts of milk were needed to provoke the reaction.

Recently, Hill et al. (33, 67, 91, 92) have even described three main groups based on the findings in 100 children with CMPA/CMPI. Besides the above defined group of immediate reactors (27% of the patients) he classified the *delayed onset reactors* into two different groups: *intermediate reactors* (53% of the patients) characterized by onset of predominantly gastrointestinal symptoms within 1 h of

milk challenge, and *late reactors* (20%) of the patients) with onset of symptoms more than 24 h after milk challenge. The symptoms in the latter group were mostly chronic cutaneous eruptions, diarrhoea or respiratory problems.

Evidence of *IgE-mediated reactions* indicated by specific IgE antibodies (positive SPT and/or RAST to CMP) was found in a total of about 50% in the study by Hill et al. (67). Although IgE-mediated reactions are predominating among immediate reactors a great deal of overlap was demonstrated. In accordance with these studies other investigators found a similar correlation between immediate reactions to food challenge and positive SPT/ positive RAST (93–95).

In a recent study (44) no significant association between symptomatology and clinical type of reaction (immediate or late) was found. However, a trend towards overrepresentation of non-IgE-mediated late reactors and a significant overweight of non-IgE-mediated gastrointestinal symptoms in part confirmed the results of previous studies (35, 67, 90, 92).

The above-mentioned differences may be ascribed to several factors such as varying age at the time of investigation (differences in composition of materials), different sample sizes, different definitions of immediate reactors and differing challenge schedules. Generally, in order to be able to compare the results of milk challenge studies in children it should be stressed that, besides symptoms and reaction time, total amount of milk protein, age and bodyweight of the patient should also be taken into account.

Interestingly, it was noted in one study (44) that 5 infants (8–26 weeks) with immediate onset reactions at initial milk challenge but without immunological evidence of IgE-sensitization to CMP (negative SPT and RAST) all at rechallenge at the age of 1 year were proven to be IgE-sensitized to CMP. This indicates that the sensitivity of these tests may be rather low in young, sensitized infants, probably due to several factors, e.g. a rather low amount of IgE-antibodies in serum and skin, a decreased reactivity of skin mast cells, an inappropriate quality of extract and/or methodological errors. In contrast, already at 12 months a good correlation was found between “immediate reactions” and the demonstration of high levels of IgE-antibodies to CMP (12/12 SPT > 3 mm and RAST class > 2 to CMP) (44) in agreement with previous reports (90, 92).

Children with *non-IgE-mediated* reactions to CMP mostly show late or delayed reactions. Evidence of the involvement of other types of immune mechanisms due to complement activation, circulating immune complexes or T-cell mediated reactions has been documented as reviewed by Bahna

(96). The clinical significance of these findings will be discussed later.

Clinical characteristics of infants with suspected but non-demonstrable CMPA/CMPI

In prospective longitudinal population studies about 60%–67% of infants suspected of having CMPA/CMPI have been disproved to suffer from this disease based on well-defined elimination/challenge procedures (39, 44, 53). In contrast to the symptomatology of infants with confirmed CMPA/CMPI, the symptomatology of infants with suspected but non-provable CMPA/CMPI has only been incompletely reported (39, 44, 53). In one study (39) it was mentioned that 7/30 infants with non confirmed CMPA/CMPI did not improve on elimination diet, whereas 23 seemed to improve on a milk free diet, but symptoms did not recur at milk challenge. However, in one study (44, 97) clinical data on 78 infants with non-provable CMPA/CMPI have been compared to those of 39 infants with confirmed CMPA/CMPI. Of these 78 infants one had lactase deficiency, three had egg allergy, 9 did not improve on milk-free diet, and 65 who apparently became symptom-free on milk withdrawal remained symptom-free after challenge (97). In infants with non-provable CMPA/CMPI 49% had cutaneous, 37% gastrointestinal, and 22% respiratory symptoms compared with the corresponding figures of 64%, 59% and 33%, respectively, in infants with confirmed CMPA/CMPI (97). The most striking difference was the occurrence of > 2 symptoms in 36/39 (92%) with CMPA/CMPI versus 32/78 (41%) ($p < 0.05$) with non-confirmed CMPA/CMPI (97). Furthermore, earlier onset of symptoms (median age 1 month) and positive SPT to CMP were predominant findings in infants with proven CMPA/CMPI (44, 97).

Treatment

Avoidance of cow's milk

The basic treatment of CMPA/CMPI is complete avoidance of CMP. Prophylactic pharmacologic agents are rarely needed. During infancy milk avoidance is easy, and a cow's milk substitute can be the only diet for infants up to 6 months of age and would ensure a milk-free diet (26, 29). In older children > 1 yr milk substitutes are usually not strictly needed, but correct avoidance of milk products may be difficult, because many commercially available foods may contain small quantities of milk proteins not listed on the labels (98). Therefore, a specialist dietitian is often needed to ensure a correct milk-free diet and sufficient nutrition. In patients with CMPA/CMPI it is usually recommended to avoid solid foods until 6 months of age and highly allergenic

foods (such as eggs, peanuts, citrusfruit, fish, chocolate and tomato) until 12 months of age (26, 29).

Cow's milk substitute products

Common commercial milk substitute formulae include soybean formula, casein hydrolysates and whey hydrolysates. In some instances heat-treated cow's milk (evaporated milk) and goat's milk have been claimed to be useful. Moreover, it has often been declared by parents that unhomogenized cow's milk is tolerated by some milk-allergic children (41), and animal studies (99, 100) gave some support to the suggestion that unhomogenized milk might be less allergenic than homogenized milk.

Soy formulae – Soy protein is reported to be at least as antigenic as cow's milk protein (101–105), and in infants with CMPA/CMPI soy protein allergy/intolerance has been recorded with frequencies of 17% (52), 20% (80), 28% (66), 35% (39), 36% (64) and 47% (140). Thus, adverse reactions to soy occur in about 17% to 47% of children sensitive to CMP. In one study (44) the patients were not treated with soy formulae and consequently the infants did not develop soy allergy during early infancy.

Casein hydrolysates – Casein hydrolysates have been widely used in CMPA/CMPI for over 40 years and are generally regarded as "hypoallergenic" (106). The casein hydrolysates are enzymatically extensively hydrolysed containing low molecular weight compounds with less than 1% of their hydrolysate over 1500 daltons. The effectiveness of these preparations in CMPA/CMPI has been documented in (39, 65, 75, 106, 107). Recently, Sampson et al. (106) proved tolerance to a casein hydrolysate in all 23 cow's milk allergic children investigated by DBPCFC. However, some instances of intolerance to casein hydrolysates have been noted (80), and a few casuistic reports on allergic reactions to casein hydrolysates have been published (108–110). In the vast majority of children with CMPA/CMPI casein hydrolysates seem safe and effective. Some disadvantages of the casein hydrolysates are low palatability and bitterness, the latter due to bitter peptide formation during proteolysis (111). Among 39 infants with documented CMPA/CMPI (44) a casein hydrolysate (Nutramigen) was offered to all until 12 months of age without observation of any allergic reactions. Only 3 infants (8%) refused to take Nutramigen (at the age of 10 months), possibly due to unpalatability? In contrast, the bitterness of extensively hydrolysed hypoallergenic formulae is rarely accepted by older infants and children.

Whey hydrolysates – Partially hydrolysed whey hydrolysates, which have been used for a few years,

contain > 20% of molecules > 1500 daltons. Several cases of allergic reactions to these products have been reported (107, 112, 113), and generally the usage of these preparations in CMPA/CMPI is not recommended as stated in a recently published position paper on hydrolysed cow's milk formulae (114). However, in infants with non-IgE-mediated reactions (CMPI), e.g. enteropathy, partially hydrolysed whey hydrolysates may seem useful (115).

Recently, a new extensively hydrolysed and ultrafiltrated whey hydrolysate has revealed "hypoallergenic" properties in "high risk" infants and infants with CMPA/CMPI comparable with those of a casein hydrolysate (116–118). Further clinical evaluation of the value of whey hydrolysates is awaited.

Heat-treated cow's milk – The effect of heat treatment on milk protein has been investigated in animal studies (119–121). In general heat treatment may destroy heat-labile milk proteins (especially BSA and BIG), and change the antigenic structure of other whey proteins, such as BLG, ALA (122). The caseins are more heat stable, but strong heat treatment (121°C for 20 min.) reduces their sensitizing capacity (120). Low pasteurization (75°C for 15 s), spray drying, evaporation and other "normal" processing procedures in the production of commercial milk products and cow's milk-based infant formulas do not eliminate, but only reduce the antigenicity and allergenicity of the milk proteins (122–124). Perhaps, boiling for 10 min may eliminate the allergenicity of the most heat-labile whey proteins (124). However, most CMPA/CMPI patients are allergic to several milk proteins (32). Therefore, boiled cow's milk will not be effective in most patients considering that Goldman et al. (32) reported that 13% of their patients reacted only against BSA. In accordance with these early results it was found that low heat treatment (pasteurization at 75°C for 15 s) did not reduce the ability of raw cow's milk to evoke allergic reactions in children with CMPA in a DBPCFC study (41).

Goat's milk – Goat's milk (evaporated goat's milk) has earlier been recommended to CMPA/CMPI patients. However, most of the proteins of goat's milk (and also sheep's milk) share identity with those of cow's milk resulting in cross-reactivity (124–126). Thus, persons with CMPA/CMPI may react at their first exposure to goat's milk. Previous experiences about successful treatment with goat's milk in CMPA/CMPI may be due to the fact that these goat's milk products were often extensively heat-treated (evaporated/boiled) leading to a reduction and perhaps elimination of the antigenicity of some of the heat-labile proteins (BSA, BIG), thus explaining their benefit in at least some patients. However,

goat's milk is deficient in certain vitamins, especially folic acid, D, C, B6 and B12, and is rich in mineral salts. Therefore, unmodified goat's milk, on several reasons, is unsuitable for feeding human infants, and should not be recommended as a milk substitute to patients with CMPA/CMPI (127).

Raw untreated and unhomogenized cow's milk

Results of studies in mice might indicate that homogenization renders the milk more allergenic "more sensitizing" (128), and more capable of inducing allergic reactions (99, 100). However, in a clinical study of children with CMPA/CMPI open challenges with unhomogenized pasteurized and raw untreated CM resulted in definite allergic reactions in 24/26 (92%) and 21/25 (84%), respectively (129). In a double-blind placebo-controlled study in milk allergic children with IgE-mediated reactions (CMPA) (41), neither raw untreated CM, pasteurized CM, nor homogenized/pasteurized CM were tolerated, disproving the opinion of many parents that raw untreated CM and unhomogenized CM are tolerated by milk-allergic children. If the latter view is claimed, one might wonder if the diagnosis of CMPA/CMPI is correct. Uncontrolled and self-appointed provocations with either unhomogenized CM or raw untreated CM in proven milk-allergic patients may be harmful and even fatal and should be warned against.

In conclusion – Only documented hypoallergenic formulae can be used in infants with CMPA/CMPI. In children with non IgE-mediated reactions (CMPI), predominantly gastrointestinal reactions, partially hydrolysed whey formulae may be useful (115), whereas extensively hydrolysed formulae should be preferred in infants with immediate reactions to CMP, mainly IgE-mediated (CMPA). The extensively hydrolysed formulae have been found substantially less antigenic and allergenic than partially hydrolysed formulae in CMPA individuals (130–135) although individuals highly sensitized to CMP may react against residual quantities of CMP even in extensively hydrolysed formulae illustrating the fact that none of the "hypoallergenic" formulae are "non-allergenic". It has been recommended that a hypoallergenic formula should fulfill the criteria of 90% clinical tolerance (with 95% confidence limits) in infants with proven IgE-mediated CMPA (114, 136). Before individual use a SPT with a fresh sample of the recommended ready-to-feed formula should be done in order to evaluate the allergenicity. If SPT is positive, then an open challenge with the chosen formula should be performed under supervision of a qualified physician, before daily usage is started (114). In case of intolerance against an extensively hydrolysed formula an amino acid-derived

elemental formula may be tolerated even in children highly allergic to CMP (137)

Prognosis

Prognosis of CMPA/CMPI

Only a few previous reports have focused on the duration of CMPA/CMPI in *prospective observational studies* of primarily *unselected infants* reporting a recovery in 45% at 1 year, 60% at 2 years (39) and a recovery in > 84% at 3 years (53). A recent Danish study (44) showed remission from CMPA/CMPI in 56% at 1 year, 77% at 2 years and 87% at 3 years confirming a good prognosis of CMPA/CMPI in infancy.

Other authors have reported on the prognosis of CMPA/CMPI in *selected groups* of infants. Kuitunen et al. (80) found an almost total recovery in infants with cow's milk-induced gastroenteropathy before 2 years of age, and in most cases recovery was obtained within 1 year. Clein in 1958 (138) reported on long-term follow-up of 206 infants with CMPA/CMPI from a general pediatric practice; he found that about 80% at 1 year, 85% at 2 years, and approximately 95% at 6 years had lost their sensitivity to CMP, and about 2% of infants with CMPA/CMPI continued to react to CMP. Hill et al. 1979 (59) found a remission rate of 12/17 (71%) at 3 years in Australian infants with CMPA/CMPI. In a Swedish study (1979) Dannaus & Johansson (90) followed 47 infants (ages between 2 weeks to 20 months at entry into the study) for a period varying between 6 months to 4 years (mean 28 months). They reported the development of a decreased sensitivity to CMP in 87% and a complete tolerance to CMP in 47%. Hill et al. 1989 (139) followed-up 47 infants with CMPA/CMPI aged 3 to 66 months. After a period of 6 to 39 months, median 16 months, 18/47 (38%) had developed tolerance to CMP. In 1990 the same Australian group (140) described the follow-up of 97 of initially 100 children with CMPA/CMPI, aged 1 to 98 months (mean 16 months at entry into the study), rechallenged with milk after 11–135 months (mean 60 months), and finally reviewed after > 5 years (mean age 99 months). Remission from CMPA/CMPI had occurred in 28% at 2 years of age, in 56% at 4 years, and in 78% at 6 years. Recently, Hill et al. (141) reported the results of 98 children (median age 24 months) followed for 2 years. Development of tolerance occurred in 15/69 (22%) of immediate reactors compared to 17/29 (59%) late reactors. Likewise, in an American study 11/29 (38%) of selected infants with CMPA (median age 3 years) became tolerant of milk over 3 years (142). The results from these studies of *selected infants* showing a lower remission rate are not comparable with those of the 3

Table 3. Percentage of children with CMPA/CMPI with reproducible adverse reactions to other individual foods

Study	Gerrard et al. 1967 b (64)	Gerrard et al. 1973 b (52)	Hill et al. 1979 b (59)	Dannaeus & Johansson 1979 a (90)		Jakobsson & Lindberg 1979 a (39)	Bishop et al. 1990 b (140)	Høst & Halcken 1990 a (44)	
No. of children	150	59	17	28 CMPA	19 CMPI	20	97	21 CMPA	18 CMPI
Duration of follow-up	–	12–36 mo	36 mo	6–48 mo mean 28 mo		24 mo	5 yr	36 mo	
Reactions to other foods Cum. prev. (%)	–	–	41	–	11	60	75	62	44
Egg	7	12	41	50	0	20	58	38	17
Soy	36	20	12	14	11	35	47	10	0
Peanut	–	–	6	0	0	0	34	5	0
Orange	7	20	0	0	0	30	35	29	17
Fish	–	–	0	23	0	15	13	5	6
Tomato	–	7	0	0	0	0	12	19	6
Banana	–	–	0	0	0	0	18	5	0
Cereals	5	8	0	11	0	10	16	10	6
Fruit	–	–	0	14	0	10	0	10	0
Beef	5	–	0	0	0	0	15	5	0
Strawberry	–	–	0	0	0	0	11	0	0
Apple	–	–	0	0	0	0	5	10	6
Nuts	–	–	0	0	0	0	5	10	6
Lamb	0	5	12	0	0	0	7	0	0
Rosehip	–	–	0	0	0	10	0	0	0
Chicken	3	2	0	0	0	0	9	0	0
Rice	5	7	0	0	0	0	0	0	0
Pear	0	0	0	0	0	0	8	0	0
Carrot	–	–	0	0	0	0	0	5	6
Cocoa	–	–	0	0	0	0	0	5	0

a Prospective observational studies of primarily unselected infants.

b Selected groups of infants with CMPA/CMPI

– Figures not given

studies of unselected infants from 0–3 years (39,44,53) showing a high remission rate, probably due to different composition of the materials. In particular the children were older at entry into the studies reporting lower remission rates from CMPA/CMPI, probably reflecting a (higher) more frequent occurrence of “low grade” transient CMPA/CMPI in young children (39, 44, 53). This observation agrees with other findings from follow-up of smaller numbers of children with CMPA/CMPI (143, 144). Only one prospective study of *unselected* infants followed from birth for development of CMPA/CMPI has reported a very low remission rate of 16/59 (27%) at 2 years of age (52). However, in that study 4/59 babies were lost to follow-up, and the remainder were followed for between 12 and 36 months. Furthermore, in that study repeated controlled milk challenges at planned intervals were apparently not performed. Thus, some factors may indicate that the real remission rate was underestimated in that study.

Interestingly, the Danish study (44) revealed a lower remission rate of 16/21 (76%) before 3 years in infants with IgE-mediated cow's milk allergy (CMPA) versus 18/18 (100%) in infants with non-IgE-mediated reactions (CMPI). Similar findings have been reported by some authors (90, 140, 141), but not by others (139). However, the latter study is not comparable to the three before mentioned due to a shorter period of follow-up.

In conclusion – Based on the results of systematic follow-up studies the prognosis of CMPA/CMPI is good, particularly in infancy and early childhood with a remission rate about 45–55% at 1 year, 60–75% at 2 years, and 85–90% at 3 years. With increasing age a lower proportion of still intolerant children will develop tolerance to CMP. Moreover, it seems clear that infants with IgE-mediated CMPA have a lower rate of recovery than infants with non IgE-mediated reactions to CMP.

Associated adverse reactions to other foods

The development of adverse reactions to other foods in children with CMPA/CMPI has been described by previous investigators (39, 52, 59, 64, 90, 140).

Table 3 shows a comparison between the frequencies of associated food intolerances from various studies (39, 44, 52, 59, 64, 90, 140). Highly allergenic foods seem to be egg (7%–58%), soy (10%–47%), peanut (5%–34%), orange (7%–30%), fish (6%–23%), tomato (6%–19%), banana (5%–18%), cereals (5%–16%), peas (10%–14%), beef (5%–15%), strawberry (11%), apple (5%–10%), nuts (5%–10%). The differences between the studies probably mainly reflect differences in diagnostic criteria, definitions, selection of patients, and in feeding habits, particularly as regards the age at introduction of certain foods, e.g. we only found a few patients with adverse reactions to soy, whereas a large proportion was identified in studies in which soy formula was used routinely in early infancy (39, 52, 64, 140). These observations support the opinion that food allergy is primarily a problem in infancy and early childhood, and most often the disease will develop in the same order as that in which the foods are introduced into the diet.

We (44) found the highest point prevalences of reproducible adverse reactions to other foods to be 9/21 (43%) in infants with CMPA and 6/18 (33%) in infants with CMPI at 18 months. The highest cumulated prevalences in the two groups were 13/21 (62%) and 8/18 (44%), respectively (44). A total remission occurred before three years in 12/21 (57%). However, as to persistence of CMPA/CMPI, IgE-sensitized children (CMPA) more often tended to remain allergic to other foods after 3 years 8/13 (62%) versus 1/8 (13%) of infants with CMPI, $P < 0.05$. These results support the findings of Dannaeus & Inganäs (144) reporting a higher frequency of associated food intolerances in infants with IgE-mediated reactions to CMP (CMPA), and in part another study (140) which showed similar results as regards persistent adverse reactions to egg, orange and peanut. Neither these two studies, nor previous studies have reported *in detail* on the clinical course of associated adverse reactions to other foods. In the Danish study (44) we also noted that persistence of adverse reactions to egg and demonstrable IgE-mediated reactions to egg (SPT ≥ 3 mm and RAST $\geq$ class 2 to egg white) in 5/8 children was associated with persistence of IgE-mediated reactions to CMP, and in 5/8 with development of inhalant allergy.

Development of allergy against inhalant allergens

Many years ago Clein (138) reported that up to 80% of infants with CMPA developed other major al-

lergic diseases (inhalant allergies) before they reached the age of puberty. Recently, Bishop et al. (140) reported from a long-term follow-up of 97 children with CMPA/CMPI (followed for a period of > 5 years (mean age at follow-up 8.25 years)) that 40% had asthma, 43% allergic rhinitis and 21% atopic eczema. They stated that exclusion of cow's milk from the diet of infants and young children with CMPA/CMPI did not prevent the subsequent development of atopic disorders, and they suggested that independent mechanisms control the development of CMPA/CMPI and other atopic diseases.

In a recent study (44) inhalant allergy developed before 3 years in 11/39 (28%) particularly against dog, cat and house dust mite, to which allergens the children had been exposed from early infancy. In 10 of 11 instances the infants belonged to the group with IgE-mediated reactions to CMPA/CMPI, and in five children persistence of IgE-mediated CMPA was associated with persistence of sensitization and allergy to egg white. These data support the findings of previous studies (145) that development of high levels of IgE antibodies to cow's milk and egg white in early infancy is often predictive of subsequent allergy to inhalants.

In conclusion – The overall prognosis, following dietary elimination of CMP, was excellent with a remission rate of 87%. However, the infants with IgE sensitization to CMP had an increased risk of persisting CMPA (24%), development of persisting allergy to other foods (particularly egg white (29%)), and, finally, inhalant allergy (48%) before 3 years of age. Long-term follow-up of our patients is needed to elucidate the late prognosis of infants with CMPA/CMPI. The increased risk for development of other persisting allergies before 3 years seems to be confined to the group of infants with evidence of an early increased IgE-response, maybe rather due to a genetic predisposition than to early CMP exposure.

Immunological aspects

Predictive capacity of immunological diagnostic tests

Adverse reactions to CMP may be due to the interaction between one or more milk proteins and one or more immune mechanisms. The symptoms may be shared with several other diseases, and the symptoms are seldom pathognomonic. Therefore, a demand for an optimal laboratory test for CMPA/CMPI has often been expressed. However, considering that any of the four basic types (I–IV) of hypersensitivity reactions may be involved, it is not surprising that no single laboratory test can distinguish individuals allergic to CMP from those who are not (96).

Immunological diagnostic tests in CMPA/CMPI have been reviewed by Savilahti (20), Hill (27), Businco et al. (146) and recently by Bahna (96,147).

Evaluating patients for CMPA/CMPI the results of an immunologic diagnostic test should be compared with the results of controlled elimination/challenge procedures, e.g. DBPCFC as the "gold standard" (36). Four issues must be considered when evaluating a diagnostic test:

- 1) *Sensitivity*: percentage of positive tests in patients with the disease;
- 2) *Specificity*: percentage of negative tests in patients without the disease;
- 3) *Predictive value of a positive test*: percentage of diseased patients among those with a positive test;
- 4) *Predictive value of a negative test*: percentage of healthy patients among those with a negative test.

Furthermore, it is important to be aware that the prevalence of a disease influences the value of a positive test, e.g. in a population with a low disease prevalence the predictive value of a positive test will be low in spite of high sensitivity and specificity. Thus, it is important exactly to define the study population in which the diagnostic test is to be used, i.e. only patients with well-defined, well characterized and reproducible symptoms should be included.

Tests for demonstration of IgE-mediated reactions, type I reactions

Skin tests

Previously, scratch test with CMP was used, but about 5% of healthy children (148) and about 12% of atopic children without CMPA/CMPI (149) showed positive to CMP. Also intracutaneous test with CMP should be avoided due to many "false positive" reactions (68%) in atopic children without CMPA/CMPI (20, 150). In later studies the value of SPT has been confirmed in identifying CMPA patients with immediate reactions mainly from the skin, the respiratory tract, and anaphylactic responses (44, 59, 92–95, 151, 152). In these studies sensitivities between 41%–100% were reported. The lowest sensitivity of 41% was found in a prospective study (44), in which the specificity was 97%, the predictive value of positive SPT 89%, and the predictive value of negative SPT 77%. These values reflect the composition of the study group with a large proportion (23/39) of infants with non-IgE-mediated reactions to CMP at the time of initial diagnosis (median 6 months). In the same study group (44) at rechallenge at 12 months a higher sensitivity and a high predictive capacity of SPT were found confirming the results of previous re-

ports of the value of SPT to CMP in older age groups (59, 92–95, 151, 152) with a higher proportion of children with IgE-mediated reactions. It should be borne in mind that differences concerning quality and purity of the applied extracts may account for widely varying results of SPT analyses. To be able to compare the results of future studies standardized extracts (153) and a well-defined proper technique should be applied as recommended by Dreborg (154).

RAST

Specific IgE demonstrated by positive RAST to CMP was seen in about 13% of children with atopic dermatitis without evidence of CMPA/CMPI (155), and in about 5% of healthy infants (156). A positive RAST to CMP was found in the majority of children with atopic dermatitis and CMPA (20). As for the SPT several studies have confirmed the value of RAST in identifying CMPA infants with mainly immediate reactions to CMP (59, 92–95, 151, 152). In 40 children with atopic dermatitis Sampson et al. (95) found the following values of RAST to CMP compared with DBPCFC: sensitivity 60%, specificity 77%, negative predictive value 93% (very useful), and a positive predictive value at 27%. Overall SPT seems to be superior to RAST as a diagnostic tool in infants with symptoms suggestive of CMPA (44, 92, 152). However, RAST can be valuable in patients with atopic dermatitis who cannot be skin tested because of their disease. Furthermore, RAST has a high negative predictive value in atopic dermatitis. As for SPT, one must be cautious about comparing results from different studies using different methods and different extracts for RAST.

Development of tolerance in children with CMPA has been associated with a decrease in specific IgE antibodies to CMP in three studies (45, 90, 142). This finding differs from the frequently reported opinion that tolerance to cow's milk and other foods develop despite the persistence of elevated levels of antigen-specific IgE antibodies (139, 144, 157, 158). In one prospective study (45) strictly withholding milk resulted in a fall in specific IgE antibodies to CMP followed by a significantly higher increase in infants with persistent CMPA after milk challenge. Therefore, the degree of IgE response seems to be a better predictor of persisting IgE-mediated cow's milk allergy than a single finding of elevated specific IgE, which can occur in normal infants without clinical disease (159).

CRIE

By the CRIE method 36 precipitates have been identified in bovine milk protein (123, 124, 161). Among the milk proteins with the strongest IgE-binding

capacity are ALA, BLG, BSA and BIG (124). Using the CRIE method (161) specific IgE antibodies to ALA, BLG, BSA, BIG and BLF were measured in 39 infants with later development of CMPA/CMPI (45). In this prospective study measurements were taken at regular intervals during the first year (at birth (CB), at 6 months and at 12 months of age). In cord blood 25/33 (76%) of the infants with later CMPA/CMPI had specific IgE-AB to one or more of the individual bovine milk proteins, an exceptionally high frequency of specific IgE in CB. No difference was found between infants with later development of CMPA (14/18) and those with later CMPI (11/15). At 6 months the frequency of specific IgE-AB was significantly higher in infants with CMPA 13/16 (81%) compared to infants with CMPI 4/15 (27%). At 12 months higher CRIE-scores (specific IgE in 32/37 (86%)) were found in infants with persisting CMPA. Thus, at birth (in CB) the very sensitive CRIE method was not able to distinguish between later CMPA and CMPI. However, due to lack of proper controls it is uncertain whether the high frequency of pos CRIE to CMP's in CB is "true" and reflecting the selection of infants with later CMPA/CMPI. At 6 and 12 months positive CRIE to specific milk proteins were associated with persistence of CMPA indicating a possible predictive capacity. The latter finding is supported by a recent study monitoring specific IgE to CAS and BLG by ELISA (142). Future studies may confirm the proposed predictive value of monitoring specific IgE to CMPs in individuals with CMPA.

Leucocyte histamine release

Sensitized blood basophils release histamine *in vitro* in the presence of the offending food antigen (reviewed 96). The food antigen reacts with specific IgE-antibodies fixed on the surface of basophils, and induces release of histamine reflecting a type I reaction. The diagnostic value of the leucocyte histamine release test in children with CMPA/CMPI seems comparable with that of RAST to CMP (162). Interestingly, investigation of histamine release from intestinal mast cells may be valuable in CMPA/CMPI patients without other signs of immunologically mediated reactions. No correlation was found between histamine release from intestinal mast cells and blood basophils (163). Further studies are needed to evaluate the significance of these tests.

Tests for demonstration of non-IgE-mediated reactions

Type II (cytotoxic) and Type III (immune complex) reactions

Type II reaction involves interaction between cell membrane-bound antigen and circulating IgG or IgM antibodies. Activation of the complement chain results in cytolysis. Activation of the complement

system probably causes the cleavage of C3a and C5a, which have anaphylatoxic activities possibly degranulating mast cells. The influence of the type II reaction in CMPA/CMPI has not been clarified.

Type III-like reactions involve fixation of complement to immune complexes between antigen and specific antibody in moderate excess of antigen. IgG (except IgG4), IgM, IgA and occasionally IgE antibodies are involved. This type of reaction is supposed to play a role in some delayed reactions to CMP, e.g. colitis, gastroenteropathy and pulmonary hemosiderosis (Heiner's syndrome). Determination of circulating immune complexes seems to be of poor diagnostic value in differentiating food sensitive patients from normal subjects (96). The formation of food antigen containing circulating immune complexes is probably a normal phenomenon reflecting the degree of antigen absorption (gut permeability) and clearance (164).

As to the pathogenesis of CMPA/CMPI, activation of the complement system was indicated in a previous study (165). However, a study with food-allergic children, who underwent double-blind challenges did not demonstrate significant changes in the levels of complement factors (166). Deposition of C3 was observed in the duodenal mucosa (167), but this observation was not confirmed (168). In view of this lack of precise information the level of complement activation by the measurement of serum complement factor split product C3d was investigated in 17 children with challenge verified CMPA/CMPI in relation to renewed milk challenge (43). Measurement of complement factor split product C3d did not reveal any indication of complement activation neither in clinically reactive children with IgE-mediated reactions (CMPA) nor in children with non-IgE-mediated reactions (CMPI). In the latter group C3d was determined up to 24 h after start of milk challenge. The degradation product C3d more precisely indicates complement activation (43, 169), whereas levels of total C3 as measured in previous studies (165, 166, 170) may fluctuate due to increased production rate or degradation. Presently, there is no convincing evidence for systemic activation of the complement system in CMPA/CMPI. However, local complement activation in the gut may take place (166) as shown in inflammatory bowel disease (170).

Further search for responsible mediators other than those of the complement system seems relevant, particularly in patients without signs of IgE-mediated milk allergy.

Type IV cellular immune reaction

Sensitized T-lymphocytes exposed to a specific antigen respond by proliferation, release of lympho-

kines, and attraction of mononuclear cells and neutrophils resulting in tissue damage. Several reports suggest that T-cell mediated reactions play a role in *delayed reactions to CMP*, mainly gastrointestinal and skin reactions (33). Cellular tests for type IV reactions include *in vitro* lymphoblast transformation test (172, 173), lymphoblastic stimulation test (174, 175), and leucocyte migration inhibition test (33, 176–179). Hill et al. (1988) demonstrated an increased production of leucocyte migration inhibitor factor (LIF) in response to ALA, BLG and alpha-CAS in clinically well-defined late reactors, but not in immediate reactors and controls (33). The same study group (180) also reported an increased *in vitro* gamma-interferon production in response to BLG-stimulation of blood mononuclear cells from patients with late reactions to CMP. Tainio & Savilathi (1990) used a BLG lymphocyte stimulation test and found among young infants (< 8 months) with immediate reactions to CMP that the majority was without specific IgE AB to CMP, but had a pos BLG lymphocyte stimulation test (175). These authors stated that no single laboratory test was sufficient to discriminate between the children who reacted to CMP and those who did not. The best combination of tests was measurement of CMP-specific IgE and the index of lymphocyte stimulation with BLG. This combination had a sensitivity of 88% (14/16), and a specificity of 67% (8/12). Comparable results were found by other Finnish investigators (186) applying a panel of five skin (including patch test) and laboratory tests in the diagnosis of CMPA/CMPI. Confirmatory studies are awaited before any conclusion concerning the value of combination of tests can be drawn. Unfortunately, these tests are relatively blood consuming, and difficult to standardize. Modern technology may improve the conditions for these tests.

The relevance of T-cell mediated reactions in CMPI with mainly gastrointestinal symptoms is also indicated by morphologic studies (181,182) showing increased activity and numbers of intraepithelial lymphocytes with T-cell characteristics in the small intestine.

Quite recently, Kondo et al. (183–185) reported food antigen – (OA and BSA)induced T-lymphocyte proliferation in children with atopic dermatitis and food allergy, and in addition an increased production of interleukin-2 and gamma-interferon was found (185). However, many technical factors affect the results of proliferation assays, and the significance of these interesting findings must await further investigations.

Specific IgG, IgM and IgA antibodies to cow's milk protein Healthy infants

Antibodies of these isotypes can be determined by different methods such as precipitation, hemagglutination, complement fixation, ELISA, and fluorescent immunosorbent test (reviewed 96). The diagnostic value of these methods in CMPA/CMPI is not clarified because formation of these AB to CMP is a normal physiological response following exposure to CMP (187–189), and high titers may rather reflect increased exposure (increased absorption) than abnormal immune reactions to cow's milk proteins. Only IgG AB to CMP were found in cord blood. Recent studies have confirmed these early reports. Thus, Tainio et al. 1988 reported (198 healthy infants) that the levels of IgG AB to CMP were highest and increased most rapidly in infants exposed to cow's milk formula before the age of 1 month. A second rise of these AB was seen after introduction of dairy milk (190). Further, partially breastfed and fully weaned babies had similar levels of these AB. In contrast, the levels of IgA and IgM AB, which were absent at birth, increased similarly in exclusively breastfed and in cow's milk formula-fed infants (190). Thus, either the amount of CMP required to elicit an IgG response may be much greater than the amount to elicit an IgA or an IgM response, or antiidiotypic AB to CMP in breast milk may play a role since maternal diet without CM did not influence the levels of IgA and IgM AB to BLG (190, 191).

Infants with CMPA/CMPI

The role of IgG and IgG subclass AB in CMPA/CMPI is controversial. Some authors have reported low titers of IgG AB to CMP (139, 194) in patients with CMPA and IgE AB to CMP, and others have found high levels of IgG AB (103, 104, 195) often in combination with high specific IgE levels (93, 175, 195). In patients with delayed reactions to CMP high titers of IgG AB to BLG (197) have been demonstrated. Also much controversy exists on the possible pathogenetic role of IgG subclass (198), and especially IgG4 in patients with CMPA/CMPI. It has been argued whether IgG4 functions as a blocking AB or as an anaphylactic AB. One study showed some evidence for a pathogenetic role of IgG4 antibodies in the provocation of both immediate and delayed reactions (199), whereas two other reports did not confirm any evidence for specific IgG4 AB playing a role in CMPA/CMPI (200, 201).

Recently, for the first time, immunologic changes including IgG AB to OA and BLG (202), IgG subclass AB to BLG (203) and IgG4 RAST to bovine whey (204) were investigated longitudinally already

from birth (cord-blood) through 6 months to 12 months in 39 infants with CMPA/CMPI and controls (45). The immunologic changes were related to initial antibody levels (including total IgE and specific IgE AB to milk proteins (as measured by CRIE)) and to development of and later remission of CMPA/CMPI (45). At any time, no single determination of specific IgG AB or IgG subclass AB to BLG or bovine whey was diagnostic of CMPA or CMPI. These results are in contrast with some earlier studies (103, 195), but agree with most recent studies (194, 200, 201). In the former study (45) decreasing levels of specific IgG from birth to 6 months probably reflect passive maternal transfer of IgG through the placenta (205–208), and withholding milk resulted in a significant fall in specific IgG AB (and IgE AB) to milk proteins followed by an increase after milk challenge, reflecting a normal physiological humoral response to antigen elimination and exposure.

Significantly higher levels of specific IgG to milk proteins in infants with persistent CMPA (45) may indicate a close relationship between the synthesis of IgE and IgG (208) and between IgE and IgG subclasses (IgG1 and IgG4) in symptomatic cow's milk allergic individuals, possibly reflecting a common genetically governed synthesis of both isotypes and/or an increased intestinal uptake and subsequent exposure to CMP. Interestingly, Finnish investigators have recently reported a relation between development of tolerance to CMP and the ability to mount a local immune response against CMP antigens, particularly in the IgA class (209).

In conclusion – Determination of specific IgG or IgG subclass AB to milk proteins is of limited diagnostic value.

Etiological and pathogenetic aspects

Introduction

As a variety of symptoms is associated with CMPA/CMPI it is not surprising that several different immunological mechanisms may be involved in the pathogenesis of the disease (31). Development of CMPA/CMPI seems to depend on several factors, especially genetic predisposition, allergen (CMP) exposure, and allergen uptake and handling. The relatively high frequency of CMPA/CMPI during infancy is suggested to be due to an incomplete mucosal barrier, increased gut permeability to large molecules, and immaturity of local and systemic immunological responses (210, 211). Furthermore human colostrum/milk facilitates maturation of the gut and provides passive protection (specific secretory IgA and additional protective factors) against bacteria and antigens (210, 212)

Distribution of individual cow's milk proteins

In developed countries cow's milk proteins are the first foreign proteins given to infants. All cow's milk proteins, more than 25, can act as allergens. Cow's milk contains 30–35 g/l of total protein, 24–28 g/l caseins (CAS) (80%), and 5–7 g/l whey proteins (20%) (213). Individual whey proteins are: BLG 2–4 g/l (9%), ALA 1–1.5 g/l (4%), proteose-peptones 0.6–1.8 g/l (4%), blood proteins: BSA 0.1–1.4 g/l (1%), BIG 0.6–1.0 g/l (2%). Because of a micellar structure casein can easily be separated from whey. The curd produced during cheese-making is primarily casein. The molecular weights of the caseins range from 25,228 for alpha-2-CAS to 11,557 for gamma-3-CAS. BLG has a molecular weight of 18,263, although it exists as a dimer at pH of milk. ALA has a molecular weight of 14,174 (213). BLG was once thought to occur only in milk from ruminants, but it has now been found in the milk of seven non-ruminant species: pig, horse, donkey, dog, dolphin, manatee and kangaroo. However, no trace of BLG was ever found in milk from mouse, rat, rabbit, guinea pig, and human. Furthermore, human milk is devoid of alpha-S1 and alpha-S2-CAS (126).

Antigenicity and immunogenicity of cow's milk proteins

Formation of specific IgE, IgA and IgM AB to CMP is a normal physiological response (188–190) and *antibodies to isolated milk proteins* (BSA, CAS, ALA, BLG and BIG) and ovalbumin have been demonstrated in normal children with the highest levels in infancy (214). Also in adults formation of specific AB to dietary antigens including milk proteins is a normal phenomenon (198). Individuals with diffuse small bowel disease and possible enhanced intestinal permeability may have higher titres of serum antibodies to milk protein (and other foods) probably only indicating increased exposure to antigen and not clinical hyperreactivity to the antigen in question (215).

Some authors have suggested the hypothesis that high specific IgG levels to CMP in cord blood (216, 217) or increased IgG/IgE ratio (144) might protect against development of CMPA/CMPI or, indicate an earlier recovery from CMPA/CMPI (144), whereas the results of Iikura et al. (218) indicated the opposite hypothesis. Recently (45), determination of specific IgG and IgG subclass AB as well as specific IgE AB in cord blood was unable to discern infants at risk for development of CMPA/CMPI. Nor, did these tests offer any predictive value concerning development of tolerance to CMP in CMPA/CMPI.

Recent reports indicate that IgE response to milk and egg protein (peaking about the age of 8–12 months) is a normal physiological event in infants

without signs of clinical disease due to these foods (159,219). In these long-term follow-up studies by Hattevig et al. only high levels of IgE AB to milk and egg protein in early infancy were predictive of later development of allergic disease.

It can be concluded that cow's milk proteins are antigenic/immunogenic in humans. The humoral immune response due to systemic immunity to foods involving formation of IgG, IgA and IgM antibodies is usually harmless. Low levels of IgE antibodies to foods may not be harmful, whereas high levels are predictive of present or later development of allergic disease.

T-cell mediated reactions in the gut have been documented in several reports (reviewed 220). Active immune responses can be detected and measured in humans, as well as in animals, whereas the phenomenon of immunological tolerance to ingested protein is best demonstrated in experimental animal studies. There is evidence, mainly based on animal studies, that T-cell mediated reactions in the small bowel mucosa cause immune mediated damage in the intestinal mucosa and malabsorption.

Allergenicity of cow's milk proteins

In cow's milk allergy the allergens are the naturally occurring proteins found in cow's milk (160, 213), although new allergenic epitopes may be revealed by technologic processing of food and/or by digestion. When allergy to cow's milk proteins has developed, the allergenicity can be demonstrated by different methods.

Oral challenge

Goldman et al. (32) orally challenged a group of infants with CMPA/CMPI: 66% reacted with BLG, 57% with CAS, 54% with ALA and 54% with BSA. Few children (13%) had reactions solely to the heat labile BSA. Lebenthal (24) reviewed the results of five studies and found that sensitivity to BLG occurred in 82%, to CAS in 43%, to ALA in 41%, to BIG in 27%, and to BSA in 18%. In children with gastroenteropathy due to CMPI Kuitunen et al. found that 5/6 responded to CAS, 1/4 to ALA, 6/7 to BLG, 1/7 to BSA and 1/5 to BIG (80).

Evidence of specific IgE to individual cow's milk proteins in patients with CMPA

Based on *skin test* Bleumink et al. (221) reported BLG the most reactive, whereas Kuitunen (80) found positive reactions to BLG, ALA and BIG.

By *RAST* to CMP's a decreasing reactivity pattern of BLG, ALA, CAS and BSA was found (222). By *RAST* or leucocyte histamine release assay CAS gave the greatest response (223).

Specific IgE identified by *CRIE* showed 36 precipitates in bovine milk protein (123, 124, 160). In sera from children with CMPA ALA, BLG, BSA and BIG showed the strongest IgE-binding capacity as well as IgE AB against 7 casein components were identified (124). In our prospective study (45) of infants with CMPA/CMPI specific IgE AB to whey proteins in cord blood were demonstrated to BSA (70%), BLG (42%) and BIG (21%). At 6 months, at the time of diagnosis, positive reactions were found in 45% to BSA, 35% to BLG, 10% to BIG, 6% to ALA and 3% to BLF. At 12 months positive *CRIE* was found in 81% to BSA, 60% to BLG, 49% to BIG, 16% to ALA, 14% to BLF. Thus, in CMPA/CMPI during the first year of life the most common whey allergen was BSA followed by BLG and BIG. The discrepancy between the latter findings and those from previous reports may be due to primarily a younger age group in our study, and methodological differences.

In conclusion – The major allergens in infants seem to be BSA, BLG and BIG, whereas in older children BLG, CAS and ALA may be more reactive. Most patients react to two or more proteins.

Lymphokine studies

Lymphokine detecting assays and lymphoblast stimulation tests have indicated a pathogenetic role of T-cell mediated reactions to individual cow milk proteins, mostly reactions to BLG, ALA and alpha-CAS (33, 96, 175, 180, 183–185).

Exposure to cow's milk proteins

Modification of cow's milk proteins

Adapted CM-based formulas – A few decades ago home-made and unadapted cow milk formulas were used for infant feeding when breast-feeding was not possible or insufficient. Nowadays, unadapted formulae are seldom used, and adapted CM-based formulae with a reduced protein concentration of about 15 g/l and a changed whey/casein ratio of 60/40 comparable with that of human milk are commonly used. In comparison cow's milk contains about 35 g/l with a whey/casein ratio of 20/80. These adapted CM-based formulas contain the same proteins as raw cow's milk (124). They are mostly processed by low pasteurization (75°C for 15 s), homogenization at 60°C and a pressure of 175 kg/cm², and spray drying/evaporation. The same procedures of low pasteurization and homogenization are used in the production of most commercial consumption milk products.

Food technology processing – Low heat treatment (low pasteurization at 75°C for 15 s) only ensures bacteriological quality of milk and does not cause

any significant reduction of the antigenicity (123, 124, 160) or the allergenicity (41). Strong heat treatment (121°C for 20 min) destroys the allergenicity of the whey proteins but only reduces that of caseins. In general, heat treatment only reduces, but does not eliminate the allergenicity of milk proteins.

No significant reduction of the allergenicity was provided by unhomogenized milk compared to neither pasteurized nor pasteurized/homogenized milk in challenge studies of infants and children with CMPA/CMPI (41, 129).

An early report suggested clinical reactivity to partially digested bovine milk proteins (224), but this suggestion could not be confirmed by other authors (225). Later, Haddad et al. (226) reported 6 patients only IgE-reactive to proteolytic digests of BLG. Schwartz et al. (227) demonstrated a higher frequency of specific IgE-antibodies to native undigested BLG than to BLG-digests in patients with suspected CMPA/CMPI. Recent studies give support to the latter findings (197). Furthermore, Jakobsson et al. have demonstrated that the duodenal juice from infants with CMPA/CMPI hydrolysed ALA, BLG and CAS as efficiently as the duodenal juice from normal infants (228). Thus, convincing evidence for an increased allergenicity of partially digested bovine milk proteins is lacking.

Absorption of cow's milk proteins

Excessive uptake of cow's milk antigens has been suggested as a causative factor in CMPA/CMPI (229), but only a few recent studies have directly addressed the question in human beings (220, 229).

Macromolecular absorption in normal infants – The intestinal permeability in preterm and term newborns to milk proteins (BSA/BLG) and to inert sugar molecules (lactulose, rhamnose) has been investigated (231, 232), and premature infants had an increased permeability. Other investigators (233) found different patterns of absorption for BLG and CAS in both preterm and term neonates possibly indicating differential antigen handling. Swedish investigators (234, 235) have developed a new method of analysing gut absorption of macro-molecules using human ALA, the dominant whey protein of human milk with a molecular weight of 14,000 daltons. They found an increased permeability in preterms and a decreasing absorption of human ALA with increasing age. In addition a delayed postnatal decrease in macromolecular absorption was demonstrated in small-for-gestational age infants (236).

Macromolecular absorption in disease states in infancy – Increased absorption of macromolecules (human ALA) has been demonstrated in children with cow's milk allergy (237), and likewise an in-

creased macromolecular absorption in infants with colic (238). Previously, Fälth-Magnusson et al. (239) using different sized PEG (PEG 400 and 1,000) reported significant changes (increase or decrease) in intestinal permeability in children with CMPA/CMPI after milk challenge.

In our study (43) of 17 children (aged 3–78 months, median 12 months) with CMPA/CMPI we investigated the uptake of BLG with ELISA (43, 240) in relation to positive milk challenge. BLG was only detectable in 5/17 children at low serum levels (below 2 ug/l). Analysis of the size distribution of the BLG by size exclusion chromatography (HPLC, 240) indicated immunoreactive material as small aggregates.

In a previous study (241) of children with coeliac disease we found BLG levels up to 100 ug/l after the intake of 10 ml of cow's milk per kg body weight in relation to gluten challenge. The low or unmeasurable BLG levels in this study may be the result of a too low dose of BLG before clinical reaction or a primary local hypersensitivity reaction in the gut mucosa. In experimental animals increased passage across the mucosa of "bystander" proteins, but not of the allergen in question has been demonstrated (242, 243) and a similar phenomenon might take place in CMPA/CMPI. The low frequency of macromolecular absorption in this study might also be explained by a large proportion of older children, in which "gut closure" has occurred. Perhaps, the above mentioned new Swedish method (237) using human ALA as a marker of macromolecular absorption is preferable when analysing intestinal permeability and macromolecular absorption in CMPA/CMPI, as no antigen reactions are to be expected against this molecule, which is a normal constituent of human milk and is taken up unaffected by local and systemic immune responses. However, as different patterns of absorption for individual cow's milk proteins may exist (233) investigation of the uptake and handling of single relevant antigens will still be of utmost interest studying the pathogenesis of CMPA/CMPI. Whether an increased macromolecular absorption is a part of an allergic constitution/heredity or is due to temporary mucosal damage is not yet clarified. Thus, this still needs further clarification including evaluation of the relevance of an increased absorption for development of clinical disease.

Macromolecular absorption in adults – More than 60 years ago Brunner & Walzer demonstrated that even the normal adult gastrointestinal tract can absorb macromolecular dietary (fish) antigens (244). They used a modified Prausnitz-Küstner test. Recently, Husby et al. (240) found, using ELISA and HPLC, that OA antigens in normal adults are taken up and can be measured in the blood in quantities of

ng/ml serum (as intact antigen or as high molecular weight immune complex). The concentration of the macromolecular proteins seems to be about 100,000 times less in the circulation (serum values) than in the provided meal.

Jackson et al. (245), using polyethylene glycols (PEG) with molecular weight 4,000 as a marker, found that the absorption was increased in all patients with eczema regardless of the presence of food allergy. Using different sized PEG (PEG 400 and PEG 1,000) these findings were not confirmed by other investigators neither in atopic mothers (246) nor in atopic children (247). Studies in adults of the uptake of macromolecules in disease states have mainly focused on dietary antigens contained in immune complexes. Thus, uptake of bovine kappa-casein in some IgA-deficient patients and some milk allergic subjects (248) has been demonstrated. Furthermore, BLG and OA within immune complexes were reported in patients with atopic eczema as well as in normals (249).

It has been recognized for more than 70 years that breast-fed infants may repeatedly react to food proteins which have passed from mother to infant via the breast milk (60, 250) giving evidence of maternal intestinal uptake of macromolecular food proteins. Thus, O'Keefe (60) and subsequently Ratner (250) were the first to demonstrate macromolecular absorption of cow's milk protein, while other early studies on this subject reported on reactions to chocolate (250), and egg (252, 253). Later clinical studies have confirmed these early findings as regards cow's milk (60, 68, 69, 254), and egg (254, 255). Recent studies have reported the presence of one of the major cow's milk allergens (221) BLG in human milk as analysed by radioimmunological methods with frequencies between 18% (256), 44% (257), 47% (258), 53% (259) and 68% (260) of nursing mothers. In one study (260) signs of CMPA/CMPI in the infants were correlated with high amounts of BLG in breast milk, but in another study (257) this was not the case.

In our study of CMPA/CMPI in exclusively breast-fed infants (40) we examined the presence of BLG by ELISA (40, 240) in human milk in relation to maternal intake of cow's milk and in relation to symptoms of the 9 infants with elimination/milk challenge verified reproducible reactions to CMP in human milk. Analysis of the size distribution was performed by HPLC in combination with ELISA (40, 240). We were able to detect BLG in 3/9 samples of human milk, (0.5–45 ng/ml), against which the infants reacted clinically, and in 1/10 control samples. There may be several reasons why we did not detect BLG in all the samples given to the infants with CMPA/CMPI. The appearance of the protein in the human milk may be slower than 4 h

after the mother's intake of milk, at which time the milk samples were collected. BLG could be present in the milk in amounts smaller than the detection limit of the assay (0.3 ng/ml), or the BLG in the breast milk may not be immunogenic in the assay. Alternatively, the infants may react to other putative allergens in the cow's milk.

Interestingly, BLG was detected with a MW corresponding to that of the monomeric protein, and no difference was observed between the milk samples given to the nine allergic infants, and the single positive control sample. In a previous study we measured BLG in sera from coeliac children in high MW fractions corresponding to the native, dimeric protein (MW 36,000) (241). The mechanisms responsible for the uptake of foreign protein from the gut to the blood and the subsequent passage to breast milk have not been clarified. Gross differences in the molecular form of the protein given with the mother's milk as compared to normal uptake seem improbable, but the quantities as such may be of importance for the development of CMPA/CMPI in these infants. Accordingly, we decided to investigate the levels and the kinetics of appearance of BLG in human milk in relation to the atopic state of the mothers. Furthermore, as the intestinal absorption of milk protein might differ not only between atopics and non atopics (245–249), but also between varying preparations of cow's milk (99, 100, 128), we decided to investigate the possible influence of homogenization.

In this study (42) 10 healthy mothers and 10 atopic mothers during 3 weeks in a cross-over design alternated their intake of milk between homogenized and unhomogenized milk each week. On Day 7 in each week consecutive milk samples were taken before and 4, 8, 12 and 24 h after a single ingestion of 500 ml of homogenized or unhomogenized milk. Detectable amounts of BLG (0.9–150 ug/l, median value 4.2 ug/l) were measured in 19/20 of the mothers (95%), in 9 of 10 atopic mothers and in all 10 of 10 non-atopic mothers. No correlation was found between the type of milk preparation (homogenized or unhomogenized) and the presence of BLG or the level of BLG in human milk. A great intra-individual and interindividual variation of BLG levels was found, and no relationship was observed between BLG levels and atopic status of the mothers. The interval between ingestion of 500 ml of milk and the maximal concentration of BLG on milk-free diet varied between 4 and 24 h, median value 8 h. All the infants had only been breastfed, and none of them showed atopic symptoms neither during the study period, nor until the age of 1 year. As opposed to previous reports with an occurrence of BLG in human milk varying between 18% and 68% (256–260), when single non-consecutive human milk

samples are analyzed, the present data (42) show that occurrence of bovine BLG in human milk is quite normal (19/20) when consecutive, continual testing of breast milk is made after the mother's consumption of normal quantities of milk. These data clearly indicate that macromolecular absorption is a normal event in practically all lactating woman.

The levels of BLG (0.9–150 ug/l) were comparable to those previously found (40, 256–260), and a great intra- and inter-individual variation was also confirmed. Our findings do not support the previous reports suggesting an increased permeability and macromolecular absorption in atopic individuals (237, 245, 249). We admit some lack of matching of the two groups, the atopic mothers being studied 1–2 weeks into their lactation, whereas the non-atopic mothers had been breast feeding for 8–19 weeks. However, a previous study (260) showed that the concentration of BLG was of the same magnitude during the whole lactation period up to 31 weeks after delivery. One might suspect that penetration of macromolecules would be higher very early in lactation. However, in the present study even in atopic mothers no correlation between early lactation and high levels of BLG in human milk was found.

No previous reports have investigated the possible influence of the type of milk preparation on the presence of bovine BLG in human milk. According to murine studies (99, 100, 128) an enhanced absorption of milk proteins from homogenized milk preparations might be expected to result in an increased transfer of milk proteins to the breast milk. Our study shows (42) that the level of bovine BLG in human milk does not differ after intake of homogenized milk compared with unhomogenized milk, pointing against an increased absorption of bovine milk protein from homogenized preparations. The present study does not allow any conclusions concerning other milk proteins. However, BLG is one of the major allergens in cow milk (221) and cow milk allergic individuals usually react against two or more milk proteins (32, 124). Future comparative studies of the levels and the kinetics of appearance of bovine caseins in human milk might be warranted for further elucidation of this field.

Time of exposure

Sensitization to food protein, e.g. milkprotein, may occur prenatally or postnatally.

Prenatal sensitization – Prenatal formation of non-specific IgE in the human foetus has been found in *in vitro* studies from the 11th week of gestation (261). Placental transfer of maternal IgE does not occur (262). In contrast, antigens may cross the placenta and elicit a *specific IgE* response in the

foetus indicated by the demonstration of specific IgE AB to various allergens, such as milk, egg, wheat, penicillin and microfilaria in cord blood (reviewed 263).

Specific CB IgE AB to milk protein has been found in 3/136 (2%) and 2/96 (2%) of unselected newborns (264, 265) by means of RAST analysis. In infants with an atopic predisposition (at least one first degree relative with atopic disease) Chandra (1989) reported RAST to CM in 9/245 cases (3.7%) (266). However, other investigators did not demonstrate CB-IgE AB to milk proteins (RAST analysis) in several hundreds of newborns (102, 205, 267–271) at high risk for development of allergy, indicating that prenatal sensitization is infrequent. In contrast, in our study (45) specific CB-IGE to BSA, BLG or BIG by means of CRIE was demonstrated in 25/33 (76%) of infants with later CMPA/CMPI, indicating a possible intrauterine sensitization. No difference was found between infants with later development of CMPA compared to those with CMPI. The discrepancy between the infrequent occurrence of specific CB-IgE of 0%–3.6% in infants with an atopic predisposition in previous studies (102, 205, 266–271), and our high frequency of 25/33 (76%) may be due to several factors, e.g. a “true” intrauterine synthesis of specific IgE to bovine protein in this selected material of newborns (39/1749) with later development of CMPA/CMPI, possible “contamination” from maternal blood (materno-fetal admixture however not indicated considering low CB-IgA values), or methodological differences. Our semiquantitative CRIE method for individual bovine milk proteins (124, 161) may be more sensitive than the RAST analysis for whole cow's milk protein used in previous studies. According to (161) the specificity of our CRIE method should be comparable to that of the RAST analysis. Unfortunately, due to exhaustion of the control sera because of other assays we did not investigate cord-blood from controls without later development of CMPA/CMPI. However, all 8 control sera at 6 months showed negative specific IGE to the same individual bovine proteins by the same CRIE method measured in the same run. Future research evaluating specific IgE AB in cord blood to individual bovine proteins and whole CMP analysed by sensitive CRIE and RAST methods is needed to elucidate the degree of intrauterine sensitization in infants with and without high risk for development of CMPA/CMPI (272).

If prenatal sensitization to CMP is really as frequent as indicated by our results in cases with later CMPA/CMPI, clinical reactions at first exposure to cow's milk in the sensitized infants might have been expected, and that did not occur (40, 46). In fact, none of the 39 infants with CMPA/CMPI in our study developed symptoms before one week, in con-

trast to the observations by some authors (39, 59, 64, 273) describing a few cases of clinical reactions to CMP at the first cow's milk-containing meal during one of the first days of life. Moreover, an immediate skin test reactivity to CM (and wheat) has been documented at 5 h of age in a newborn, whose mother did not react to these allergens (274).

Recent prospective studies have investigated the possible effect of maternal abstention from high potential allergens (such as milk and egg) during late pregnancy on the development of atopic diseases in "high risk" infants in infancy (275–277). No beneficial effect was demonstrated either in these studies or in studies where the maternal diet without milk and egg was extended to the lactating period (65, 277, 278). In one of these studies (278) the authors reported a significant effect. However, the main influence on development of atopic eczema in that study appeared to be associated with postnatal exposure to cow's milk.

In conclusion – No convincing protective effect on childhood allergy in high risk infants has been observed by maternal food allergen avoidance during pregnancy or during pregnancy and later lactation. Furthermore, no specific IgE antibodies to egg white (ovomucoid) or BLG were detected in cord sera from high risk newborns of mothers with a high daily intake of these foodstuffs (271, 279). Thus, if intrauterine sensitization occurs, it seems to be infrequent or weak and the clinical relevance remains to be clarified.

Postnatal sensitization – Postnatal sensitization to foods and reproducible clinical reactions to foods are principally a problem of early infancy. Neonatal exposure to and uptake of food proteins may be increased due to several factors such as an incomplete mucosal barrier, increased gut permeability to macromolecules, and immaturity of local and systemic immunologic responses (cellular and especially humoral immune response) as reviewed by Walker (210) and Strobel (220). As intestinal permeability is more pronounced in preterm neonates and causes increased absorption of macromolecular bovine milk proteins in preterm neonates an increased frequency of CMPA/CMPI among these infants might be expected. However, in our population based study group (40, 44, 46) of 1749 newborns only 1 premature infant developed CMPA, making up a frequency of about 1.1% among preterms. Recently, Lucas et al. (280) reported the incidence of CMPA/CMPI among 777 preterm infants in a randomized prospective study to be 34/777 (4.4%) based on a carefully obtained clinical history. Apparently, CMPA/CMPI does not occur more frequently in preterm infants.

Amount of exposure

High dose antigen exposure – In infants fed CM-based formula the antigenic load of CMP may be appreciable during the first year amounting to about 10–15 g per day or about 3.6–5.5 kg per year when considering that normal CM based formula contains about 15 g/l. Cow's milk feeding *per se* has been reported to increase intestinal permeability in newborns (281), this possibly enhancing the effect of the physiological IgA deficiency, including the poorly developed response of secretory IgA in the neonatal period (282, 283). The net effect may be an increased antigen absorption including an "overstimulation" of the immune system resulting in IgE sensitization (284). However, human colostrum/human milk facilitates maturation of the gut and may provide a passive protection against absorption of antigens (282, 283) (specific secretory IgA AB). In a Finnish study (285) of seven infants low colostrum IgA seemed to be associated with development of CMPA. Another paper reported that possible allergic symptoms in 11 of 57 exclusively breast-fed infants were significantly correlated with low levels of total IgA and specific IgA AB to whole CM and casein (257). The clinical diagnosis of these infants was not verified by controlled challenge procedures. Recent studies have not been able to confirm these findings (286). In infants without atopic predisposition fed cow's milk formula salivary IgA increased significantly between 1 and 9 months, and significant increases in serum IgE occurred between 3 and 6 months as opposed to breast-fed infants. None of the 25 infants developed allergic disease (287). Investigation of salivary IgA in symptomatic infants with CMPA/CMPI compared with controls might elucidate the significance of a possible protective role of salivary IgA.

A possible protective effect of breast-feeding on the development of atopic diseases may be due to either 1) avoidance of "high dose" of CMP or 2) a positive protective effect of human milk. For over 50 years it has been debated, whether breast-feeding can influence the risk of atopic diseases, and numerous studies have been conducted to test this hypothesis. Unfortunately, the results of these studies have been contradictory, probably because of varying design, size and quality of these studies (reviews of earlier prospective studies (263)). Common factors apparent in studies reporting benefit of breast-feeding were 1) prolonged breast-feeding (> 4–6 mo), and 2) delayed solid food introduction. Common to all the studies nonsupportive of breast-feeding benefits was 1) rather brief period of breast-feeding < 3–4 mo (frequently < 6 weeks), and 2) early solid food introduction.

In a review 12 standards were developed pertaining to exposure, outcome and statistical analysis for studies on the influence of early feeding on the development of atopic diseases (288), and it was concluded that no important differences were found between positive and negative studies. However, it does not seem correct to equalize the worth of standards as non-reliance on prolonged maternal recall (retrospective studies), sufficient duration and exclusivity of breast-feeding, separate analysis of children at high risk for development of atopy, severity of outcome, age at onset of outcome, and assessment of dose response effect. In my opinion, it appears questionable to rely on retrospective studies in which data on early feeding are based on prolonged maternal recall. Thus, presumably, exclusively breast-fed infants have very often (20–85%) been given inadvertent supplements of, e.g., cow's milk based formula (40, 254, 263). A protective effect of breast-feeding may only be seen and confirmed in a selected group of children at high-risk for development of atopy, unless the study population is very large. Ideally, randomization to breast- or bottle-feeding should be done, but it seems unreasonable (and impossible) to allocate infants to bottle-feeding, if nursing is possible. However, if nursing is not established randomization to different formulae should be done (cow's milk based formula or hypoallergenic formula), and careful attention should be paid to the following demands on study design: prospective study, selected high risk sample, sufficient duration of breast-feeding (> 4–6 mo), documentation of exclusive breastfeeding, late introduction of solid foods (4–6 mo), blind ascertainment of feeding history, blind evaluation of atopic disease, strictly well-defined diagnostic criteria, control for confoundings (environmental control), documentation of compliance, and adequate statistical power (large samples).

Recent prospective clinical trials in high risk infants on the possible protective effect of breast-feeding or hypoallergenic protein hydrolysates (when breastfeeding not possible) in combination with avoidance of intake of CMP and solid foods during > 4 months have shown a significant reduction of the incidence and severity of atopic dermatitis (266, 269, 289, 290), a reduction in recurrent wheezing (116, 269) and a reduced incidence of CMPA/CMPI (116, 117, 280, 290, 291, 292) during the first 1–2 years of life. Due to some weaknesses concerning study design further proof and comparative studies between different hypoallergenic formulae (extensively versus partially hydrolysed) are needed (114).

Further evidence of an association between early CM formula feeding and CMPA/CMPI is obtained from our prospective observational study of a 1-year

cohort of 1749 newborns from whom a careful dietary history was taken from the first day of life (40, 46). Infants with CMPA/CMPI were fed cow's milk formula during the first month of life significantly more often (46) than infants in the study population (46). These data support the results of previous studies (47, 293) indicating an association between early CM formula feeding and later development of CMPA/CMPI in keeping with the opinion that increased allergenic exposure is supposed to enhance sensitization. Data on early neonatal supplements of cow's milk formula during the first 3 days in the newborn nursery (40) revealed that 88% of 1539 infants received supplement of cow's milk formula. All 39 infants with CMPA/CMPI belonged to that group (39/1539). None of 210 neonates without supplements of cow's milk formula developed CMPA/CMPI (0/210) ($0.02 < P < 0.05$). Furthermore, daily CM formula feeding during the first month of life seemed to be associated with an increased risk of primarily CMPI (46). The differences between early feeding patterns in infants with CMPA and infants with CMPI may indicate different pathogenetic mechanisms in CMPA (mainly breast-fed) and CMPI (mainly daily CM formula fed). However, it is important to consider that all infants with CMPA had also been given supplements (40–840 ml) of CM formula neonatally (40, 46).

Previous authors (273, 294) have reported some infants with "minimal exposure" to CM had higher levels of IgE against milk and more manifestations of CMPA/CMPI than infants who had been fed substantial volumes of cow's milk. The same authors stated that these observations supported the hypothesis proposed by Jarret (based on animal experiments in rats) that regular exposure to "large" allergen doses induces tolerance, whereas "small" allergen doses may rather sensitize (predisposed) individuals (295–297). However, it can be argued whether "small" amounts of 40 ml cow's milk formula contain a small allergen dose. In fact, it can be calculated that 40 ml of CM formula (15 g protein per liter, whey/casein ratio: 6/4) contain 0.9 g protein, and as regards the major allergen BLG the content will be approximately 0.36 g corresponding to 360,000,000 ng. This amount of BLG is enormous compared with the low amounts provided by human milk (0.9–150 ng/ml, (42)). It can be calculated that the content of BLG in 40 ml CM formula (about 360,000,000 ng) is equal to the amount provided by about 7–21 years nursing 1 l of human milk/day! Thus, it can be concluded that such "small amounts" of CM formula inadvertently given to newborns really contain enormous amounts of CM proteins compared with the "minute amounts" in breast milk.

The fact that none of 210 exclusively breast-fed infants without any neonatal supplements of cow's

milk formula developed adverse reactions to cow's milk protein (40), may suggest that early neonatal intake of cow's milk formula is a condition of sensitization to cow's milk protein and later allergic reactions to CM proteins, also in otherwise exclusively breast-fed infants, in whom subsequent exposure to the minute amounts of CM proteins in human milk may act as a booster dose eliciting allergic manifestations. Possibly, it may be more deleterious to give such formulas occasionally rather than regularly to newborns with an atopic predisposition ("high risk") considering the animal experiment by Jarret (297). The findings of one report (298, 299) seemed to indicate that continuous early feeding with CM formula during the first days of life might reduce the incidence of "atopic symptoms" until 18 months in infants with a family history of atopic disease. At later follow-up (292) at 4–6 yr no convincing effect of early CM feeding was found, and in fact CMPA/CMPI had only occurred in children early fed CM formula. Due to some weaknesses of the design of that study (e.g. lack of strict diagnostic criteria, several observers, and small sample) the significance of the results should be interpreted with caution. However, the hypothesis is interesting and calls for prospective randomized studies in "high risk" infants.

Low-dose antigen exposure – It has often been supposed that sensitization to minute quantities of CM protein in human milk may occur (61, 69, 254, 256–260, 273). In high-risk infants a potential beneficial effect of maternal hypoallergenic diet during lactation has also been studied with conflicting results, some positive (289, 300, 301) showing a reduced incidence of atopic dermatitis, but others negative (271, 277). Thus, the clinical importance of a possible sensitization via protein in human milk may seem questionable. The development of allergy may depend more on the exquisite immune responsiveness of certain predisposed individuals to specific antigens than on the amount of antigen. This suggestion is supported by findings by Cant et al. (254), who found that the frequency and the concentration of egg protein in human milk were similar in mothers of infants with and without atopic dermatitis. Furthermore, our demonstration (43) of BLG in human milk in 19 of 20 mothers (irrespective of atopic status or not) has shown that almost all newborn children under normal living conditions, where the mother consumes about 0.5 l milk daily, are exposed to bovine BLG. Considering the low incidence of cow's milk allergy (about 2–3%) during the first year of life, one might suppose that these small amounts induce tolerance to CMP rather than sensitization and subsequent allergic manifestations. If our (45) previously discussed finding of a very high frequency (76%) of prenatal sensitization to CM

protein as indicated by pos CRIE-AB in CB of infants with later development of CMPA/CMPI is reliable and later confirmed, one might speculate the prenatal sensitization to CMP to be a weak intrauterine "priming" inducing a low IgE response, which may be "boosted" postnatally, in particular neonatally, by high doses of CMP and subsequently followed by development of clinical reactions to CMP.

Discussing potential ways of sensitization to CMP it is important to remember that sensitization to foods, also during exclusive breastfeeding, may be due to "unknown breaks" in the dietary regimen, inhalation of food proteins, or contact with contaminated hands. Thus, one should consider that just 1 ml of ordinary cow's milk contains 35 mg of protein, equal to about 3 mg of BLG (or 3,000 ug) corresponding to the amount provided by about 2 months nursing 1 l human milk/day suggesting an amount of 45–50 ug BLG/l human milk!

Atopic predisposition

Among infants with CMPA/CMPI a family history of atopy (mother, father and/or sibling) was found in 19/39 (49%) versus 32% in the background population, and double parental atopic heredity in 9/39 (23%) versus 3% in the background population ($P < 0.001$) (44, 88). Although a tendency towards a higher frequency in CMPA infants no significant differences concerning elevated CB IgE (> 0.5 kU/l), atopic predisposition or double parental atopic predisposition were found among the 16 infants with CMPA and the 23 infants with CMPI at the time of diagnosis. Double parental heredity or single heredity combined with elevated cord blood IgE ("high risk") was found in 15/39 (38%) versus 73/1749 (4.2%) ($P < 0.001$) (44, 88, 116). At the time of diagnosis 9/16 (56%) CMPA infants and 6/23 (26%) CMPI infants belonged to the "high risk" group. At 12 months according to the revised classification the corresponding figures were 10/21 (48%) and 5/18 (28%), respectively. Fifteen of a total of 73 (21%) "high risk" babies developed CMPA/CMPI. These data confirm the previously reported high frequencies of atopic predisposition among first degree relatives reported by other investigators varying between 73%–80% (32), 70% (39), and 41% (66). Moreover, Gerrard et al. (64) calculated that the risk of an infant developing CMPA/CMPI is greatest if an older sibling had the same disease; in these circumstances the risk that a subsequent baby will develop CMPA/CMPI is 1:3. In our study a family history (at least one parent/or sibling) of CMPA/CMPI was only present in 4 cases (10.2%) in accordance with Ventura et al. (66) (10.8%).

Infants with a pronounced atopic predisposition seem to have a clearly increased risk for devel-

opment of CMPA/CMPI, in particular in case of early exposure to CMP and lack of breastfeeding. Such infants may have a primary immunoregulatory defect (302), which can be identified by elevated CB-IgE, high levels of specific IgE, low numbers of T-cells (303), disturbed (increased) ratio of T-helper/T-suppressor cells (304, 305) or decreased function of T-suppressor cells (304–306). Thus, early cow's milk feeding in infants with low numbers of T-cells was associated with increased IgE-levels and an increased risk of allergic symptoms in infancy (303, 306). Breast-feeding may modulate the immune response to CMP in several ways, via humoral or cellular immune reactivity, and it has been suggested (307) that a high content of gamma-linolenic acid in breast-milk might have an allergy-protective effect via influence on T-lymphocytes and IgE synthesis. This hypothesis is supported by a recent study of Businco et al. (308) reporting low levels of long-chain polyunsaturated fatty acids in human milk from mothers of children with newly developed atopic dermatitis. Controlled prospective studies of atopic mothers and their babies with regard to development of atopy and CMPA/CMPI are warranted to test this hypothesis.

References

- PIRQUET C VON: Allergie. Münch Med Wochenschr 1906: 53: 214.
- ISHIZAKA K: The identification and significance of gamma E. In: DIXON FJ, FISHER DW, eds. The biology of immunologic diseases. Sunderland: Sinauer Associates, 1983: 13–23.
- COOMBS RRA, GELL PGH: Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: GELL PGH, COOMBS RRA, LACHMANN PJ, eds. Clinical aspects of immunology. Oxford: Blackwell Scientific, 1975: 761–82.
- HANIFIN JM, RAJKA G: Diagnostic features of atopic dermatitis. Acta Dermatol Venerol 1980; 92 (suppl.): 44–47.
- CHABOT R. Pediatric Allergy. New York: McGraw-Hill, 1951.
- O'KEEFE ES. The history of infant feeding II. Seventeenth and eighteenth centuries. Arch Dis Child 1953; 28: 232–40.
- HAMBURGER F. Biologisches über die Eiweisskörper der Kuhmilch und über Säuglingsernährung. Wien Klin Wochenschr 1901; 14: 1202–4.
- SCHLOSSMANN A, MORO E. Zur Kenntnis der Arteigenheit der verschiedenen Eiweisskörper der Milch. Münch Med Wochenschr 1903; 14: 597–98.
- FINKELSTEIN H. Kuhmilch als Ursache akuter Ernährungsstörungen bei Säuglingen. Monatsschr Kinderheilk 1905; 4: 65–72.
- MORO E. Kuhmilchpräzipitin im Blute eines 4 1/2 Monate alten Atrophikers. Münch Med Wochenschr 1906; 53: 214.
- WERNSTEDT W. Zur Frage der Kuhmilchidiosynkrasie im Säuglingsalter. Monatsschr Kinderheilk 1910; 9: 25–26.
- HERTZ P. Cow's milk idiosyncrasy in infants (in Danish). Ugeskr Laeger 1914; 76: 971–8.
- HALBERSTADT R. Über Idiosynkrasie der Säuglinge gegen Kuhmilch. Arch Kinderheilk 1911; 55: 105–21.
- TALBOT FB. Idiosyncrasy to cow's milk: its relation to anaphylaxis. Boston Med Surg J 1916; 175: 409–10.
- SCHRICKER H. Beitrag zu der "Kuhmilchidiosynkrasie". Arch Kinderheilk 1921; 62: 332–41.
- VENDEL S. Cow's milk idiosyncrasy in infants. Acta Paediatr Scand 1948; 35 (suppl. 5): 1–37.
- HANSEN P. Urticaria provoked by inhalation of milk protein (in Danish). Ugeskr Laeger 1938; 100: 226.
- ROTHER MEYER A. Cow's milk idiosyncrasy (in Danish). Nord Med 1943; 17: 256.
- FLENSBORG EW. On Benadryl treatment of allergic diseases in children. Especially in cases of idiosyncrasy to cow's milk in infants (in Danish). Ugeskr Laeger 1947; 109: 639–42.
- SAVILAHTI E. Cow's milk allergy. Allergy 1981; 36: 73–88.
- HAMBURGER RN. Introduction: a brief history of food allergy with definitions of terminology in food intolerance. In: Hamburger RN, ed. Food intolerance in infancy. New York: Raven Press, 1989: 1–6.
- GOLDSTEIN GB, HEINER DC. Clinical and immunological perspectives in food sensitivity. J Allergy Clin Immunol 1970; 46: 270–91.
- FREIER S. Paediatric gastrointestinal allergy. Clin Allergy 1973; 3 (suppl.): 597–618.
- LEBENTHAL E. Cow's milk protein allergy. Ped Clin North Am 1975; 22: 827–33.
- MAY CD, BLOCK SA. A modern clinical approach to food hypersensitivity. Allergy 1978; 33: 166–88.
- BAHNA SL, GANDHI MD. Milk hypersensitivity II. Practical aspects of diagnosis, treatment and prevention. Allergy 1983; 50: 295–301.
- HILL DJ. Cow's milk allergy in infants: some clinical and immunologic features. Ann Allergy 1986; 57: 225–8.
- WALKER-SMITH JA. Milk intolerance in children. Clin Allergy 1986; 16: 183–90.
- BAHNA SL. Milk allergy in infancy. Ann Allergy 1987; 59: 131–6.
- WILSON NW, HAMBURGER RN. Allergy to cow's milk in the first year of life and its prevention. Ann Allergy 1988; 61: 323–7.
- BAHNA SL. Pathogenesis of milk hypersensitivity. Immunol Today 1985; 6: 153–5.
- GOLDMAN AS, ANDERSON JR DW, SELLERS WA, SAPERSTEIN S, KNIKER WT, HALPERN SR. Milk allergy I. Oral challenge with milk and isolated milk proteins in allergic children. Pediatrics 1963; 32: 425–43.
- HILL DJ, BALL G, HOSKINGS CS. Clinical manifestations of cow's milk allergy in childhood. I. Association with *in vitro* cellular immune responses. Clin Allergy 1988; 18: 469–79.
- WALKER-SMITH J. Cow's milk protein intolerance. Transient food intolerance of infancy. Arch Dis Child 1975; 50: 347–50.
- FORD RP, HILL DJ, HOSKING CS. Cow's milk hypersensitivity: immediate and delayed onset clinical patterns. Arch Dis Child 1983; 58: 856–62.
- BOCK SA, SAMPSON HA, ATKINS FM, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988; 82: 986–99.
- HILL DJ, HOSKING CS. Some limitations of double-blind, placebo-controlled food challenges in young children. Letter. J Allergy Clin Immunol 1991; 87: 136–7.
- KRASILNIKOFF PA, GULDMUND-HØYER E, MOLTKE HH. Diagnostic value of disaccharide tolerance tests in children. Acta Paediatr Scand 1975; 64: 693–8.

39. JACOBSSON O, LINDBERG T. A prospective study of cow's milk protein intolerance in Swedish infants. *Acta Paediatr Scand* 1979; 68: 853-9.
40. HØST A, HUSBY S, ØSTERBALLE O. A prospective study of cow's milk allergy in exclusively breast-fed infants. Incidence, pathogenetic role of early inadvertent exposure to cow's milk formula, and characterization of bovine milk protein in human milk. *Acta Paediatr Scand* 1988; 77: 663-70.
41. HØST A, SAMUELSSON E-G. Allergic reactions to raw, pasteurized and homogenized/pasteurized cow milk: a comparison. *Allergy* 1988; 43: 113-8.
42. HØST A, HUSBY S, HANSEN LK, 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: 383-7.
43. HUSBY S, HØST A, TEISNER B, SVEHAG S-E. Infants and children with cow milk allergy/intolerance. Investigation of the uptake of cow milk protein and activation of the complement system. *Allergy* 1990; 45: 547-51.
44. HØST A, HALKEN S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990; 45: 587-96.
45. HØST A, HUSBY S, GJESING B, LARSEN JN, LØWENSTEIN H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy. *Allergy* 1992; 47: 218-29.
46. HØST A. Importance of the first meal on the development of cow's milk allergy and intolerance. *NER Allergy Proc* 1991; 12: 227-32.
47. STINTZING G, ZETTERSTRÖM R. Cow's milk allergy, incidence and pathogenetic role of early exposure to cow's milk formula. *Acta Paediatr Scand* 1979; 68: 383-7.
48. COLLINS-WILLIAMS C. The incidence of milk allergy in pediatric practice. *J Pediatr* 1956; 48: 39-47.
49. BACHMAN KD, DEES SC. Milk allergy I. Observations on incidence and symptoms in "well" babies. *Pediatrics* 1957; 20: 393-9.
50. JOHNSTONE DE, DUTTON AM. Dietary prophylaxis of allergic disease in children. *N Engl J Med* 1966; 274: 715-9.
51. HALPERN SR, SELLARS WA, JOHNSON RB, ANDERSON DW, SAPERSTEIN S, REISCH JS. Development of childhood allergy in infants fed breast, soy, or cow milk. *J Allergy Clin Immunol* 1973; 51: 139-51.
52. GERRARD JW, MACKENZIE JWA, GOLUBOFF N, GARSON JZ, MANINGAS CS. Cow's milk allergy. Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* 1973; (suppl 234): 1-21.
53. BOCK SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics* 1987; 79: 683-8.
54. BIERING-SØRENSEN F, HILDEN J, BIERING-SØRENSEN K. Recent increase in breast-feeding. *Acta Paediatr Scand* 1978; 67: 665-6.
55. STÅHLBERG M-R. Breast-feeding, cow milk feeding and allergy. *Allergy* 1985; 40: 612-5.
56. VITORIA JC, SOJO A, RODRIGUEZ-SORIANO J. Changing pattern of cow's milk protein intolerance. *Acta Paediatr Scand* 1990; 79: 566-7.
57. HIDE DW, GUYER BM. Cow's milk intolerance in Isle of Wight infants. *Br J Clin Pract* 1983; 37: 285-7.
58. SCHRANDER JJP, BOGART JPH, FORGET PP, SCHRANDER-STUMPEL CTRM, KUITEN RH, KESTER ADM. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr* 1993; 152: 640-4.
59. HILL DJ, DAVIDSON GP, CAMERON DJS, BARNES GL. The spectrum of cow's milk allergy in childhood: clinical, gastroenterological and immunological studies. *Acta Paediatr Scand* 1979; 68: 847-52.
60. O'KEEFE ES. The relation of food to infantile eczema. *Boston Med Surgery J* 1920; 183: 569-73.
61. GERRARD JW. Allergy in breast fed babies to ingredients of breast milk. *Ann Allergy* 1979; 42: 69-72.
62. DELÈZE G, NUSSLE D. L'intolérance aux protéines du lait de vache chez l'enfant. Revue de 30 cas. *Helv Paediatr Acta* 1975; 30: 135-49.
63. NAVARRO J, OMANGE U, MOUGENOT J-F, BAUDON J-J, FONTAINE J-L, POLONOVSKI C, LAPLANE R. L'intolérance digestive aux protéines du lait de vache chez le nourrisson. Etude clinique de 42 cas. *Arch Fr Pediatr* 1975; 32: 773-86.
64. GERRARD JW, LUBOS MC, HARDY LW, HOLMLUND BA, WEBSTER BE. Milk allergy: clinical picture and familial incidence. *Canad Med Ass J* 1967; 97: 780-5.
65. ZEIGER RS, HELLER S, MELLON M, 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.
66. VENTURA A, GRECO L, BALLI F, et al. Cow's milk allergy in the first year of life. An Italian collaborative study. *Acta Paediatr Scand Suppl* 1988; 348: 1-14.
67. HILL DJ, FIRER MA, SHELTON MJ, HOSKING CS. Manifestations of milk allergy in infancy: clinical and immunological findings. *J Pediatr* 1986; 109: 270-6.
68. JACOBSSON I, LINDBERG T. Cow's milk as a cause of infantile colic in breast fed infants. *Lancet* 1978; II: 437-9.
69. JACOBSSON I, LINDBERG T. Cow's milk proteins cause infantile colic in breast-fed infants: a double-blind crossover study. *Pediatrics* 1983; 71: 268-71.
70. LOTHE L, LINDBERG T, JACOBSSON I. Cow's milk formula as a cause of infantile colic: a double-blind study. *Pediatrics* 1982; 70: 7-10.
71. LOTHE L, LINDBERG T. Cow's milk whey protein elicits symptoms of infantile colic in formula-fed infants: a double-blind crossover study. *Pediatrics* 1989; 83: 262-6.
72. KAHN A, MOZIN MJ, CASIMIR G, MONTAUK I, BLUM D. Insomnia and cow's milk allergy in infants. *Pediatrics* 1985; 76: 880-4.
73. KAHN A, FRANCOIS G, SOTTIAUX M, et al. Sleep characteristics in milk intolerant infants. *Sleep* 1988; 291-7.
74. KAHN A, MOZIN BA, REBUFFAT E, SOTTIAUX BA, MULLER MF. Milk intolerance in children with persistent sleeplessness: a prospective double blind crossover evaluation. *Pediatrics* 1989; 84: 595-603.
75. GRYBOSKI JD. Gastrointestinal milk allergy in infants. *Pediatrics* 1967; 40: 354-62.
76. POWELL GK. Milk- and soy-induced enterocolitis of infancy. Clinical features and standardization of challenge. *J Pediatr* 1978; 93: 553-60.
77. KUITUNEN P, VISAKORPI JK, HALLMAN N. Histopathology of duodenal mucosa in the malabsorption syndrome induced by cow's milk. *Ann Paediatr* 1965; 205: 54-63.
78. LOEB H, VAINSEL M, CADRANEL S, et al. Malabsorption and villus atrophy in four infants: relationship to cow's milk intolerance. *Acta Paediatr Scand* 1971; 60: 372-3.
79. FONTAINE JL, NAVARRO J. Small intestinal biopsy in cow's milk protein allergy in infancy. *Arch Dis Child* 1975; 50: 357-62.

80. KUITUNEN P, VISAKORPI JK, SAVILAHTI E, PELKONEN P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 1975; 50: 351-6.
81. CALDWELL JH, SHARMA HM, HURTUBISE PE, COLWELL DL. Eosinophilic gastroenteritis in extreme allergy. *Gastroenterology* 1979; 77: 560-4.
82. AGERTOFT A, HUSBY S, HØST A. Intestinal perforation in a two-year-old child with eosinophilic gastroenteritis. *Acta Paediatr Scand* 1991; 80: 389-91.
83. HEINER DC, SEARS JW, KNIKER WT. Multiple precipitins to cow's milk in chronic respiratory disease. A syndrome including poor growth, gastrointestinal symptoms, evidence of allergy, iron deficiency anemia, and pulmonary hemosiderosis. *Am J Dis Child* 1962; 103: 634-54.
84. WILSON JF, LAHEY ME, HEINER DC. Studies on iron metabolism. V. Further observations on cow's milk-induced gastrointestinal bleeding in infants with iron-deficiency anemia. *J Pediatr* 1974; 84: 335-44.
85. WHITFIELD MF, BARR BD. Cow's milk allergy in the syndrome of thrombocytopenia with absent radius. *Arch Dis Child* 1976; 51: 337-43.
86. EGGER J, CARTER CM, WILSON J, TURNER MW, SOOTHILL JF. I migraine food allergy? A double-blind controlled trial of oligoantigenic diet treatment. *Lancet* 1983; 2(8355): 865-9.
87. HEINER DC, WILSON JF. Delayed immunologic food reactions. *NER Allergy Proc* 1986; 7: 520-6.
88. HALKEN S, HØST A, HUSBY S, HANSEN LG, ØSTERBALLE O, NYBOE J. Recurrent wheezing in relation to environmental risk factors in infancy. A prospective study of 276 infants. *Allergy* 1991; 46: 507-14.
89. GERRARD JW, SHENASSA M. Food allergy: two common types as seen in breast and formula fed babies. *Ann Allergy* 1983; 50: 375-9.
90. DANNAEUS A, JOHANSSON SGO. A follow-up study of infants with adverse reactions to cow's milk. I. Serum IgE, skin test reactions and RAST in relation to clinical course. *Acta Paediatr Scand* 1979; 68: 377-82.
91. HILL DJ, FORD RPK, SHELTON MH, HOSKING CS. A study of 100 infants and young children with cow's milk allergy. *Clin Rev Allergy* 1984; 2: 125-42.
92. HILL DJ, DUKE AM, HOSKING CS, HUDSON IL. Clinical manifestations of cow's milk allergy in childhood. II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988; 18: 481-90.
93. DANNAEUS A, JOHANSSON SGO, FOUCARD T, OMAN S. Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 1977; 66: 31-7.
94. BOCK SA, LEE WY, REMIGIO L, HOLST A, MAY CD. Appraisal of skin tests with food extracts for diagnosis of food hypersensitivity. *Clin Allergy* 1978; 8: 559-64.
95. SAMPSON HA, ALBERGO R. Comparison of results of skin tests, RAST and double blind, placebo controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984; 74: 26-33.
96. BAHNA SL. Diagnostic tests for food allergy. *Clin Rev Allergy* 1988; 6: 259-84.
97. HØST A. The relevance of history for the diagnosis of cow's milk allergy. A prospective study in Danish infants. In: HARMS HK, WAHN U, eds. *Food allergy in infancy and childhood*. Berlin: Springer Verlag, 1988: 125-31.
98. GERN JE, YANG E, EVRARD HM, SAMPSON HA. Allergic reactions to milk-contaminated nondairy products. *N Eng J* 1991; 324: 976-9.
99. POULSEN OM, HAU J, KOLLERUP J. Effect of homogenization and pasteurization on the allergenicity of bovine milk analysed by a murine anaphylactic shock model. *Clin Allergy* 1987; 17: 449-58.
100. POULSEN OM, NIELSEN BR, BASSE A, HAU J. Comparison of intestinal anaphylactic reactions in sensitized mice challenged with untreated bovine milk and homogenized bovine milk. *Allergy* 1990; 45: 321-6.
101. EASTHAM EJ, LICHAUCO T, GRADY MI, WALKER WA. Antigenicity of infant formulas: role of immature intestine on protein permeability. *J Pediatr* 1978; 93: 561-4.
102. KJELLMAN N-IM, JOHANSSON SGO. Soy versus cow's milk in infants with a biparental history of atopic disease: development of atopic disease and immunoglobulins from birth to 4 years of age. *Clin Allergy* 1979; 9: 346-58.
103. MAY DC, REMIGIO L, BOCK SA. Usefulness of measurements of antibodies in serum in diagnosis of sensitivity to cow milk and soy proteins in early childhood. *Allergy* 1980; 35: 301-10.
104. MAY CD, FOMON SJ, REMIGIO L. Immunologic consequences of feeding infants with cow milk and soy products. *Acta Paediatr Scand* 1982; 71: 43-51.
105. MISKELLY FG, BURR ML, VAUGHAN-WILLIAMS E, FEHILY AM, BUTLAND BK, MERRET TG. INFANT FEEDING AND ALLERGY. *ARCH DIS CHILD* 1988; 63: 388-93.
106. SAMPSON HA, BERNHISEL-BROADBENT J, YANG E, SCANLON SM. Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediatr* 1991; 118: 520-5.
107. ZEIGER RS. Prevention of food allergy in infancy. *Ann Allergy* 1990; 65: 430-47.
108. LIFSCHITZ CH, HAWKINS HK, GUERRA C, BYRD N. Anaphylactic shock due to cow's milk protein hypersensitivity in a breast-fed infant. *J Pediatr Gastroenterol Nutr* 1988; 7: 141-4.
109. BOCK SA. Probable allergic reactions to casein hydrolysate formula. Letter. *J Allergy Clin Immunol* 1990; 84: 84: 272.
110. SAYLOR JD, BAHNA SL. Anaphylaxis to casein hydrolysate formula. *J Pediatr* 1991; 118: 71-4.
111. GUIGOZ Y, SOLMS J. Bitter peptides, occurrence and structure. *Chemi Senses Flavor* 1976; 2: 71-84.
112. ELLIS MH, SHORT JA, HEINER DC. Anaphylaxis after ingestion of a recently introduced hydrolyzed whey protein formula. *J Pediatr* 1991; 118: 74-7.
113. BUSINCO L, CANTANI A, LONGHI AL, GIAMPIETRO PG. Anaphylactic reactions to cow's milk whey protein hydrolysate (Alfa-Re, Nestle) in infants with cow's milk allergy. *Ann Allergy* 1989; 62: 333-5.
114. BUSINCO L, DREBORG S, EINARSSON R, et al. Hydrolysed cow's milk formulae. Allergenicity and use in treatment and prevention. An ESPACI position paper. *Pediatr Allergy Immunol* 1993; 4: 101-111.
115. WALKER-SMITH JA, DIGEON B, PHILLIPS AD. Evaluation of a casein and a whey hydrolysate for treatment of cow's milk sensitive enteropathy. *Eur J Pediatr* 1989; 149: 68-71.
116. 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: 545-53.
117. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Preventive effect of feeding high risk infants a casein hydrolysed formula or an ultrafiltrated whey hydrolysed formula. A prospective, comparative clinical study. *Pediatr Allergy Immunol* 1993; 4: 173-81.
118. HALKEN S, HØST A, HANSEN LG, ØSTERBALLE O. Safety of a new ultrafiltrated whey hydrolysate formula in children with cow milk allergy: a clinical investigation. *Pediatr Allergy Immunol* 1993; 4: 53-9.
119. McLAUGHLAN P, ANDERSON KJ, WIDDOWSON EN, COOMBS RRA. Effect of heat on the anaphylactic sensitiz-

- ing capacity of cow's milk, goat's milk, and various infant formula fed to guineapigs. *Arch Dis Child* 1981; 56: 165-71.
120. KILSHAW PJ, HEPPEL LMJ, FORD JE. Effects of heat treatment of cow's milk and whey on the nutritional quality and antigenic properties. *Arch Dis Child* 1982; 57: 843-7.
121. HEPPEL LMJ, CANT AJ, KILSHAW PJ. Reduction in the antigenicity of whey proteins by heat treatment: a possible strategy for producing a hypoallergenic infant milk formula. *Br J Nutrition* 1984; 51: 29-36.
122. JOST R. Physicochemical treatment of food allergens: application to cow's milk proteins. In: REINHARDT D, SCHMIDT E, eds. *Food allergy*. New York: Raven Press, 1988: 187-97.
123. HANSSON LÅ, MÅNSSON I. Immune electrophoretic studies of bovine milk and milk products. *Acta Paediatr* 1961; 50: 484-90.
124. GJESING B, ØSTERBALLE O, SCHWARTZ B, WAHN U, LØWENSTEIN H. Allergen-specific IgE antibodies against antigenic components in cow milk and milk substitutes. *Allergy* 1986; 41: 51-6.
125. CRAWFORD LV, GROGAN FT. Allergenicity of cow's milk proteins IV. Relationship to goat's milk proteins as studied by serum-agar precipitation. *J Pediatr* 1961; 50: 347.
126. RIBADEAU-DUMAS B. Structure and variability of milk proteins. In: BARTH CA, SCHLIMME E, eds. *Milk proteins. Nutritional, clinical, functional and technological aspects*. Darmstadt: Steinkopff Verlag, 1988: 112-23.
127. COVENEY J, DANTON-HILL I. Goat's milk and infant feeding. *Med J Austr* 1985; 143: 508-10.
128. NIELSEN BR, POULSEN OM, HAU J. Reagin production in mice: effect of sensitization with untreated bovine milk and homogenized bovine milk. *In vivo* 1989; 3: 271-4.
129. HANSEN LG, HØST A, ØSTERBALLE O. Allergic reactions to unhomogenized milk and raw milk (in Danish). *Ugeskr Laeger* 1987; 149: 909-11.
130. OLDAEUS G, BJÖRKSTEN B, EINARSSON R, KJELLMAN N-I M. Antigenicity and allergenicity of cow's milk hydrolysates in tended for infant feeding. *Pediatr Allergy Immunol* 1991; 2: 156-60.
131. RUGO E, WAHL R, WAHN U. How allergenic are hypoallergenic infant formulae? *Clin Exp Allergy* 1992; 22: 635-39.
132. OLDAEUS G, BRADLEY CK, BJÖRKSTEN B, KJELLMAN N-I M. Allergenicity screening of "hypoallergenic milk-based formulas". *J Allergy Clin Immunol* 1992; 90: 133-35.
133. RAGNO V, GIAMPIETRO PG, BRUNO G, BUSINCO L. Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy. *Eur J Pediatr* 1993; 152: 760-2.
134. AMONETTE MS, ROSENFELD SI, SCHWARTZ RH. Serum IgE antibodies to cow's milk proteins in children with differing degrees of IgE-mediated cow's milk allergy: analysis by immunoblotting. *Pediatr Asthma Allergy Immunol* 1993; 7: 99-109.
135. MÄKINEN KILJUNEN S, SORVA R. Bovine B-lactoglobulin levels in hydrolysed protein formulas for infant feeding. *Clin Exp Allergy* 1993; 23: 287-91.
136. KLEINMAN RE, BAHNA S, POWELL GF, SAMPSON HA. Use of infant formulas in infant with cow milk allergy. A review and recommendations. *Pediatr Allergy Immunol* 1991; 2: 146-55.
137. SAMPSON HA, JAMES JM, BERNHISEL-BROADBENT J. Safety of an amino-acid derived infant formula in children allergic to cow milk. *Pediatrics* 1992; 463-2.
138. CLEIN NW. Cow's milk allergy in infants and children. *Int Arch Allergy* 1958; 13: 245-56.
139. HILL DJ, FIRER MA, BALL G, HOSKING GS. Recovery from milk allergy in early childhood. Antibody studies. *J Pediatr* 1989; 114: 761-6.
140. BISHOP JM, HILL J, HOSKING CS. Natural history of cow milk allergy. Clinical outcome. *J Pediatr* 1990; 116: 862-7.
141. HILL DJ, FIRER MA, BALL G, HOSKING CS. Natural history of cow's milk allergy in children: immunological outcome over 2 years. *Clin Exp. Allergy* 1993; 23: 124-31.
142. JAMES JM, SAMPSON HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. *J Pediatr* 1992; 121: 371-7.
143. BOCK SA, LEE W-Y, REMIGIO LK, MAY CD. Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978; 62: 327-34.
144. DANNAEUS A, INGANÄS MA. Follow-up study of children with food allergy. Clinical course in relation to serum IgE- and IgG-antibody levels to milk, egg and fish. *Clin Allergy* 1981; 11: 533-9.
145. HATTEVIG G. Sensitization and type I allergy in infants and children. Linköping University Medical Dissertations No. 285, Linköping, Sweden 1989.
146. BUSINCO L, BENINCORI N, CANTANI A. The spectrum of food allergy in infancy and childhood. *Ann Allergy* 1986; 57: 213-8.
147. BAHNA SL. The diagnostic dilemma of milk allergy. *Ann Allergy* 1989; 63: 475-6.
148. BUCKLEY RH, DEES SC. Nutritional and antigenetic effects of two bovine milk preparations in infants. *J Pediatr* 1966; 69: 238-45.
149. BACHMANN KD, DEES SC. Milk allergy. II. Observations on incidence and symptoms of allergy to milk in allergic infants. *Pediatrics* 1957; 20: 400-7.
150. GOLDMAN AS, SELLARS WA, HALPERN SP, ANDERSON DW, FURLOW TE, JOHNSON CH, JR. Milk allergy II. Skin testing of allergic and normal children with purified milk proteins. *Pediatrics* 1963; 32: 572-9.
151. AAS K. The diagnosis of hypersensitivity to ingested foods. Reliability of skin prick testing and the radioallergosorbent test with different materials. *Clin Allergy* 1978; 8: 39-50.
152. SAMPSON HA. IgE-mediated food intolerance. *J Allergy Clin Immunol* 1988; 81: 495-504.
153. NØRGAARD A, SKOV PS, BINDSLEV-JENSEN C. Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils. *Clin Exp Allergy* 1992; 22: 940-7.
154. DREBORG S. The skin prick test, methodological studies, and clinical applications (Medical dissertation 239). Linköping, Sweden: University of Linköping, 1987.
155. BONIFAZI E, GAROFALO L, MONTERISI A, MENEGHINI CL. Food allergy in atopic dermatitis: experimental observations. *Acta Derm Venereal (Stockh.)* 1978; 58: 349-53.
156. BJÖRKSTEN F, SAARINEN UM. IgE antibodies to cow's milk in infants fed breast milk and milk formulae. *Lancet* 1978; 2: 624-5.
157. BOCK SA. The natural history of food sensitivity. *J Allergy Clin Immunol* 1982; 69: 173-9.
158. SAMPSON HA, MCCASKILL CM. Food hypersensitivity in atopic dermatitis: evaluation of 113 children. *J Pediatr* 1985; 107: 669-75.
159. HATTEVIG G, KJELLMAN B, JOHANSSON SGO, BJÖRKSTEN B. Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. *Clin Allergy* 1984; 14: 551-9.
160. LØWENSTEIN H, BJERRUM OJ, WEEKE E, WEEKE B. Characterization of bovine whey proteins by crossed immunoe-

- lectrophoresis. *Scand J Immunol* 1975; 4 (suppl 2): 155-61.
161. WEEKE B, SØNDERGAARD J, LIND P, AUKRUST L, LØWENSTEIN H. Crossed radioimmuno-electrophoresis (CRIE) for identification of allergens and determination of the antigenic specificities of patients' IgE. *Scand J Immunol* 1983; 17 (suppl.10): 265-72.
 162. PRAHL P, KRASILNIKOF P, STAHL SKOV P, NORN S. Basophil histamine release in children with adverse reactions to cow milk. Comparison with RAST and skin prick test. *Allergy* 1988; 43: 442-8.
 163. NOLTE H, SCHIØTZ PO, KRUSE A, STAHL SKOV P. Comparison of intestinal mast cell and basophil histamine release in children with food allergic reactions. *Allergy* 1989; 44: 554-65.
 164. PAGANELLI R, QUINTI I, D'OFFIZI P, PAPETTI C, CARINI C, AIUTI F. Immune complexes in food allergy: a critical reappraisal. *Ann Allergy* 1987; 59: 157-61.
 165. MATTHEWS TS, SOOTHILL JF. Complement activation after feeding in children with cow milk allergy. *Lancet* 1970; ii: 893-5.
 166. MARTIN ME, GUTHRIE LA, BOCK SA. Serum complement changes during double-blind food challenges in children with a history of food sensitivity. *Pediatrics* 1984; 73: 532-7.
 167. SHINER M, BALLARD J, SMITH ME. The small-intestinal mucosa in cow milk allergy. *Lancet* 1975; i: 136-40.
 168. PERRKIÖ M. Immunohistochemical study of intestinal biopsies from children with atopic eczema due to food allergy. *Allergy* 1980; 35: 573-80.
 169. BRANDSLUND I, SIERSTED HC, SVEHAG S-E, TEISNER B. Double-decker rocket immunoelectrophoresis for direct quantitation of complement C3 split products with C3d specificities in plasma. *J Immunol Methods* 1981; 44: 63-70.
 170. INGANÄS M, JOHANSSON SGO, DANNAEUS A. A method for estimation of circulating immune complexes after oral challenge with ovalbumin. *Clin Allergy* 1980; 10: 293-302.
 171. HALSTENSEN TS, MOLLNES TE, BRANDTZAEG P. Persistent complement activation in submucosal blood vessels of active inflammatory bowel disease: immunohistochemical evidence. *Gastroenterology* 1989; 97: 10-19.
 172. ENDRE L, OSVATH P. Antigen-induced lymphoblast transformation in the diagnosis of cow's milk allergic diseases in infancy and early childhood. *Acta Allergol* 1975; 30: 34-42.
 173. SCHEINMANN P, GENDREL D, CHARLAS J, et al. Value of lymphoblast transformation test in cow's milk protein intolerance. *Clin Allergy* 1976; 6: 515-21.
 174. BAUDON JJ, MOUGENOT JF, DIDRY JR. Lymphoblastic stimulation test with food proteins in digestive intolerance to cow's milk and in infant diarrheas. *J Ped Gastroenterol Nutr* 1987; 6: 244-51.
 175. TAINO V-M, SAVILAHTI E. Value of immunologic tests in cow milk allergy. *Allergy* 1990; 45: 189-96.
 176. MINOR JD, TOLBER SG, FRICK OL. Leukocyte inhibition factor in delayed-onset food allergy. *J Allergy Clin Immunol* 1980; 66: 314-21.
 177. ASHKENAZI A, LEVIN S, IDAR D, et al. *In vitro* cell mediated immunologic assay for cow's milk allergy. *Pediatrics* 1980; 66: 399-402.
 178. KHOSHOO V, BHEN MK, ARORA NK, et al. Leukocyte migration inhibition in cow's milk protein intolerance. *Acta Paediatr Scand* 1986; 75: 308-12.
 179. VANTO T, SMOGORZEWSKA EM, VIANDER M, et al. Leukocyte migration inhibition test in children with cow milk allergy. *Allergy* 1987; 42: 612-8.
 180. HILL DJ, BALL G, HOSKING CS, WOOD PR. Gamma-interferon production in cow milk allergy. *Allergy* 1993; 48: 75-80.
 181. PHILLIPS AD, RICE SJ, FRANCE NE, WALKER-SMITH JA. Small intestinal intraepithelial lymphocyte levels in cow's milk protein intolerance. *Gut* 1979; 20: 509-12.
 182. KOSNAI I, KUITUNEN P, SAVILAHTI E, RAPOLA J, KÖHEGYI J. Cell kinetics in the jejunal crypt epithelium in malabsorption syndrome with cow's milk protein intolerance and in coeliac disease. *Gut* 1980; 21: 1041-46.
 183. KONDO N, AGATA H, FUKUTOMI O, et al. Lymphocyte responses to food antigens in patients with atopic dermatitis who are sensitive to foods. *J Allergy Clin Immunol* 1990; 86: 253-60.
 184. AGATA H, KONDO N, FUKUTOMI O, et al. Effect of elimination diets on food-specific IgE antibodies and lymphocyte proliferative to food antigens in atopic dermatitis patients exhibiting sensitivity to food allergens. *J Allergy Clin Immunol* 1993; 91: 668-79.
 185. KONDO N, FUKUTOMI O, AGATA H, et al. The role of T lymphocytes in food-sensitive atopic dermatitis. *J Allergy Clin Immunol* 1993; 91: 658-68.
 186. RÄSÄNEN L, LEHTO M, REUNALA T. Diagnostic value of skin and laboratory tests in cow's milk allergy/intolerance. *Clin Exp Allergy* 1992; 22: 385-390.
 187. ROTHBERG RM, FARR RS. Anti-bovine serum albumin and antialpha lactalbumin in the serum of children and adults. *Pediatrics* 1965; 35: 571-88.
 188. KLETTER B, GERY I, FREIER S, DAVIES AM. Immune responses of normal infants to cow milk. I. Antibody type and kinetics of production. *Int Arch Allergy* 1971; 40: 656-66.
 189. KLETTER B, GERY I, FREIER S, DAVIES AM. Immune responses of normal infants to cow milk. II. Decreased immune reactions in initially breast-fed infants. *Int Arch Allergy* 1971; 40: 667-74.
 190. TAINO V-M, SAVILAHTI E, ARSOMAA P, SALMENPERÄ L, PERHEENTUPA J, SILMES A. 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 breastfeeding. *Acta Paediatr Scand* 1988; 77: 807-11.
 191. MELLANDER L, CARLSSON B, HANSON LA. Secretory IgA and IgM antibodies to *E. coli* O and poliovirus type I antigens occur in amniotic fluid, meconium and saliva from newborns. Neonatal immune response without antigenic exposure - a result of anti-idiotypic induction. *Clin Exp Immunol* 1986; 63: 551-61.
 192. FALTH-MAGNUSSON K, KJELLMAN N-IM, MAGNUSSON K-E. Antibodies IgG, IgA and IgM to food antigens during the first 18 months of life in relation to feeding and development of disease. *J Allergy Clin Immunol* 1988; 81: 743-9.
 193. HARRIS MC, KOLSKI GB, CAMPBELL DE, DEUBER C, MARCUS M, DOUGLAS SD. Ontogeny of the antibody response to cow milk proteins. *Ann Allergy* 1989; 63: 439-43.
 194. FIRER MA, HOSKING CS, HILL DJ. Humoral immune response to cow's milk in children with cow's milk allergy. *Int Arch Allergy Appl. Immunol* 1987; 84: 173-7.
 195. BÜRGIN-WOLFF A, SIGNER E, FRIESS HM, BERGER R, BIRBAUMER A, JUST M. The diagnostic significance of antibodies to various cow's milk proteins (Fluorescent Immunosorbent test). *Eur J Pediatr* 1980; 133: 17-24.
 196. FÄLLSTRÖM SP, AHLSTEDT S, HANSON LÅ. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl. Immunol* 1978; 56: 97-105.

197. FÄLLSTRÖM SP, AHLSTEDT S, CARLSSON B, LÖNNERDAL B, HANSON LÅ. Serum antibodies against native, processed and digested cow's milk proteins in children with cow's milk protein intolerance. *Clin Allergy* 1986; 16: 417-23.
198. HUSBY S. Dietary antigens. Uptake and humoral immunity in man. *APMIS* 1988 (suppl.): 96: 1-40. Thesis.
199. RAFFI AE, PETERS SM, HARRIS N, BELLANTI JA. Diagnostic value of IgG4 measurements in patients with food allergy. *Ann Allergy* 1989; 62: 94-9.
200. BJÖRKSTÉN B, AHLSTEDT S, BJÖRKSTÉN F, CARLSSON B, FÄLLSTRÖM SP, JUNTUNEN K, KAJOSAARI M, KOBER A. Immunoglobulin E and immunoglobulin G4 antibodies to cow's milk in children with cow's milk allergy. *Allergy* 1983; 38: 119-24.
201. BURKS AW, WILLIAMS LW, CASTEEL HB, FIEDOREK SC, CONNAUGHTON CA. Antibody response to milk proteins in patients with milk-protein intolerance documented by challenge. *J Allergy Clin Immunol* 1990; 85: 921-26.
202. HUSBY S, SCHULTZ LARSEN F, HYLTOFT PETERSEN P, SVEHAG S-E. Humoral immunity to dietary antigens in atopic dermatitis I. Biotin-avidin amplified ELISA-analysis for serum IgE antibodies. *Allergy* 1986; 41: 379-385.
203. HUSBY S, JENSENIUS JC, SVEHAG S-E. ELISA-quantification of IgG-subclass antibodies to dietary antigen. *J Immunol Methods* 1985; 82: 321-31.
204. HEDLIN G, GRAFF-LONNEVIG V, HEILBORN H, LILJA G, NORRLIND K, PEGELOW K-O, SUNDIN B, LÖWENSTEIN H. Immunotherapy with cat- and dog-dander extracts II. *In vivo* and *in vitro* immunologic effects observed in a 1-year double-blind placebo study. *J Allergy Clin Immunol* 1986; 77: 488-96.
205. DANNAEUS A, JOHANSSON SGO, FOUCARD T. Clinical and immunological aspects of food allergy in childhood. II. Development of allergic symptoms and humoral immune response to foods in infants of atopic mothers during the first 24 months of life. *Acta Paediatr Scand* 1978; 67: 497-504.
206. FÄLTH-MAGNUSSON K, OMAN H, KJELLMAN N-IM. Maternal abstinence from cow milk and egg in allergy risk pregnancies. *Allergy* 1987; 42: 64-73.
207. HATTEVIG G, KJELLMAN B, SIGURS B, GRUDSINSKY E, HED J, BJÖRKSTÉN B. The effect of maternal avoidance of eggs, cow's milk, and fish during lactation on the development of IgE, IgG, and IgGA antibodies in infants. *J Allergy Clin Immunol* 1990; 85: 108-115.
208. LILJA G, MAGNUSSON CGM, OMAN H, JOHANSSON SGO. Serum levels of IgG subclasses in relation to IgE and atopic disease in early infancy. *Clin Exp Allergy* 1990; 20: 407-13.
209. ISOLAURI E, SUOMALAINEN H, KALLA M, JALONEN T, SOPPI E, VIRTANEN E, ARVILOMMI H. Local immune response in patients with cow milk allergy: follow-up of patients retaining allergy or becoming tolerant. *J Pediatr* 1992; 120: 9-15.
210. WALKER WA. Pathophysiology of intestinal uptake and absorption of antigens in food allergy. *Ann Allergy* 1987; 59: 7-16.
211. SCOTT H, BRANDTZAEG P. Pathogenesis of food protein intolerance. *Acta Paediatr Scand* 1989; suppl. 351: 48-52.
212. JUTO P, HOLM S. Gliadin specific and cow's milk protein specific IgA in human milk. *J Pediatr Gastroenterol Nutr* 1992; 15: 159-62.
213. SWAISGOOD HE. Characteristics of edible fluids of animal origin: milk. In: Fennema OR, ed., *Food chemistry*. New York: Marcel Dekker, 1985: 791-827.
214. MAY CD, REMIGIO L, FELDMAN J, BOCK SA, CARR RI. A study of serum antibodies to isolated milk proteins and ovalbumin in infants and children. *Clin Allergy* 1977; 7: 583-95.
215. LERNER A, ROSSI T, PARK B, ALBINI B, LEBENTHAL E. Serum antibodies to cow's milk proteins in pediatric inflammatory disease. *Acta Paediatr Scand* 1989; 78: 384-9.
216. CASIMIR G, GOSSART B, VIS HL, DUCHATEAU J. Antibody against betalactoglobulin (IgG) and cow's milk allergy. *J Allergy Clin Immunol*. 1985; 75: 206.
217. CASIMIR GJA, DUCHATEAU J, CUVELIER P, VIS HL. Maternal immune status against beta-lactoglobulin and cow's milk allergy in the infant. *Ann Allergy* 1989; 63: 517-9.
218. IKURA Y, AKIMOTO K, ODAJIMA Y, AKAZAWA A, NAGAKURA T. How to prevent allergic disease. I. Study of specific IgE, IgG and IgG4 antibodies in serum of pregnant mothers, cord blood and infants. *Int Arch Allergy appl. Immunol* 1989; 88: 250-2.
219. HATTEVIG G, KJELLMAN B, BJÖRKSTÉN B. Common food proteins and inhalants in the first 7 years of life. *Clin Allergy* 1987; 17: 571-8.
220. STROBEL S. Developmental aspects of food allergy. In: REINHARDT D, SCHMIDT E, eds. *Food allergy*. New York: Raven Press. 1988: 99-115.
221. BLEUMINK E, YOUNG E. Identification of the atopic allergen in cow's milk. *Int Arch Allergy Appl Immunol* 1968; 34: 521-543.
222. BALDO BA. Milk allergies. *Aust J Dairy Technol* 1984; 39: 120-8.
223. WAHN U, GANSTER G. Cow's milk proteins as allergens. *Eur J Pediatr* 1982; 138: 94.
224. COOKE RA. Protein derivatives as factors in allergy. *Ann Int Med* 1942; 16: 71-80.
225. BLOOM S, MARKOW H, REDNER B. Studies in food sensitivity. II. The effect of protein digestion on antigenicity of foods as determined by skin test and clinical food trials. *J Allergy* 1953; 24: 64-71.
226. HADDAD ZH, KALRA V, VERMA S. IgE antibodies to peptic-tryptic digests of betalactoglobulin: significance in food hypersensitivity. *Ann Allergy* 1979; 42: 369-71.
227. SCHWARTZ HR, NERURKAR LS, SPIES JR, SCANLON RT, BELLANTI JA. Milk hypersensitivity: RAST studies using new antigens generated by pepsin hydrolysis of beta-lactoglobulin. *Ann Allergy* 1980; 45: 242-5.
228. JAKOBSSON I, LINDBERG T, BENEDIKTSSON B. In vitro digestion of cow's milk proteins by duodenal juice from infants with various gastrointestinal disorders. *J Pediatr Gastroenterol Nutr* 1982; 1: 183-91.
229. WALKER WA, ISSELBACHER KH. Uptake and transport of macromolecules by the intestine-possible role in clinical disorders. *Gastroenterology* 1974; 67: 531-50.
230. BOCK SA, REMIGIO LK, GORDON B. Immunochemical localization of proteins in the intestinal mucosa of children with diarrhea. *J Allergy Clin Immunol* 1983; 72: 262-8.
231. ROBERTSON DM, PAGANELLI R, DINWIDDIE R, LEVINSKY RJ. Milk antigen absorption in the preterm and term neonate. *Arch Dis Child* 1982; 57: 369-72.
232. BEACH RC, MENZIES IS, CLAYDEN GS, SCOPES JW. Gastrointestinal permeability changes in the preterm neonate. *Arch Dis Child* 1982; 57: 141-5.
233. MÜLLER G, BERNSAU I, MÜLLER W, WEISBARTH-RIEDEL E, NATZSCHKA J, RIEGER CHL. Cow's milk protein antigens and antibodies in the serum of premature infants. *J Pediatr* 1986; 109: 869-72.
234. JAKOBSSON I, LINDBERG T, LOTHE L, et al. Human alpha-lactalbumin as a marker of macromolecular absorption. *Gut* 1986; 27: 1029-34.
235. AXELSSON I, JAKOBSSON I, LINDBERG T, et al. Macromolecular absorption in preterm and term infants. *Acta Paediatr Scand* 1989; 78: 532-37.

236. BOEHM G, JACOBSSON J, MÄNSSON M, RÄIHÄ NCR. Macromolecular absorption in small-for-gestational-age infants. *Acta Paediatr* 1992; 81: 864-7.
237. JUVONEN P, JAKOBSSON J, LINDBERG T. Macromolecular absorption and cow's milk allergy. *Arch Dis Child* 1990; 65: 300-3.
238. LOTHE L, LINDBERG T, JAKOBSSON I. Macromolecular absorption in infants with infantile colic. *Acta Paediatr Scand* 1990; 79: 417-21.
239. FÄLTH-MAGNUSSON K, KJELLMAN NIM, ODELRAH H, SUNDQUIST T, MAGNUSSON KE. Gastrointestinal permeability in children with cow's milk allergy: effect of milk challenge and sodium cromoglycate as assessed with polyethylene glycols (PEG 400 and PEG 1000). *Clin Allergy* 1986; 16: 543-55.
240. HUSBY S, JENSENIUS JC, SVEHAG S-E. Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution and relation of uptake to levels of specific antibodies. *Scand J Immunol* 1985; 22: 83-92.
241. HUSBY S, FOGED N, HØST A, SVEHAG S-E. Passage of dietary antigen into the blood of children with coeliac disease. Quantification and size distribution of absorbed antigens. *Gut* 1987; 1062-72.
242. TOLO K, BRANDTZAEG P, JONSEN J. Mucosal penetration of antigen in the presence or absence of serum-derived antibody. *Immunology* 1977; 33: 733-43.
243. BLOCH KJ, WALKER WA. Effect of locally induced intestinal anaphylaxis on the uptake of a bystander antigen. *J Allergy Clin Immunol* 1981; 67: 312-6.
244. BRUNNER M, WALZER M. Absorption of undigested proteins in human beings: the absorption of unaltered fish proteins in adults. *Arch Intern Med* 1928; 173: 42-6.
245. JACKSON PG, LESSOF MH, BAKER RWR, SERRET J, McDONALD DM. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1982; i: 1285-6.
246. FÄLTH-MAGNUSSON K, KJELLMAN N-IM, SUNDQUIST T, MAGNUSSON KE. Gastrointestinal permeability in atopic and non-atopic mothers, assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000). *Clin Allergy* 1985; 15: 565-70.
247. FÄLTH-MAGNUSSON K, KJELLMAN NIM, MAGNUSSON KE, SUNDVIST T. Intestinal permeability in healthy and allergic children before and after sodium-chromoglycate treatment assessed with different sized polyethyleneglycols (PEG 400 and PEG 1000). *Clin Allergy* 1984; 14: 277-86.
248. CUNNINGHAM-RANDLES C. The identification of specific antigens in circulating immune complexes by an enzyme-linked immunosorbent assay: detection of bovine kappa-casein IgG complexes in human sera. *Eur J Immunol* 1982; 11: 504-9.
249. PAGANELLI R, LEVINSKY RJ, ATHERTON DJ. Detection of specific antigen within circulating immune complexes: validation of the assay and its application to food antigen-antibody complexes formed in healthy and food-allergic subjects. *Clin Exp Immunol* 1981; 46: 44-53.
250. RATNER B. Certain aspects of eczema and asthma in infancy and childhood from the standpoint of allergy. *Med Clin North Am* 1922; 6: 815-30.
251. TALBOT FB. Eczema in childhood. *Med Clin North Am* 1918; 1: 985-96.
252. SHANNON WR. Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs. *Am J Dis Child* 1921; 22: 223-31.
253. DONNALLY HH. The question of the elimination of foreign protein (egg-white) in woman's milk. *J Immunol* 1930; 19: 15-40.
254. CANT A, MARSDEN RA, KILSHAW PJ. Egg and cow's milk hypersensitivity in exclusively breast fed infants with eczema, and detection of egg protein in breast milk. *Br Med J* 1985; 291: 932-5.
255. MATSUMARA T, KUROMI T, OGURI M, et al. Egg sensitivity and eczematous manifestations in breast-fed newborns with particular reference to intrauterine sensitization. *Ann Allergy* 1975; 35: 221-9.
256. STUART CA, TWISELTON R, NICHOLAS MK, HIDE DW. Passage of cow's milk protein in breast milk. *Clin Allergy* 1984; 14: 533-5.
257. MACHTINGER S, MOSS R. Cow's milk allergy in breast-fed infants: the role of allergen and maternal secretory IgA antibody. *J Allergy Clin Immunol* 1986; 77: 341-8.
258. JACOBSSON I, LINDBERG T, BENEDIKTSSON B, HANSSON BG. Dietary bovine-lactoglobulin is transferred to human milk. *Acta Paediatr Scand* 1985; 74: 342-5.
259. KILSHAW PJ, CANT AJ. The passage of maternal dietary proteins into human breast milk. *Int Arch Allergy Appl Immunol* 1985; 75: 8-15.
260. AXELSSON I, JACOBSSON I, LINDBERG T, BENEDIKTSSON B. Bovine beta-lactoglobulin in the human milk. A longitudinal study during the whole lactation period. *Acta Paediatr Scand* 1986; 75: 702-7.
261. MILLER DL, HIRVONEN T, GITLIN D. Synthesis of IgE by the human conceptus. *J Allergy Clin Immunol* 1973; 52: 182-8.
262. STEVENSON DD, ORGEL HA, HAMBURGER RN, REID RT, RICHARDSON OE. Development of IgE in newborn human infants. *J Allergy Clin Immunol* 1972; 48: 61-72.
263. ZEIGER RS, HELLER S, MELLON M, O'CONNOR R, HAMBURGER RN. Effectiveness of dietary manipulation in the prevention of food allergy in infants. *J Allergy Clin Immunol* 1986; 78: 224-38.
264. MICHEL FB, BOUSQUET P, GREILLIER P, et al. Comparison of cord blood immunoglobulin E and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1980; 65: 422-30.
265. DELESSESSE G, SARFATI M, LANG G, SEHON AH. Prenatal and neonatal synthesis of IgE. *Monogr Allergy* 1983; 18: 83-95.
266. CHANDRA RK, SINGH G, STRIDHARA B. Effect of feeding whey hydrolysate, soy and conventional cow milk formulas on incidence of atopic disease in high risk infants. *Ann Allergy* 1989; 63: 102-6.
267. CRONER S, KJELLMAN NIM, ERICKSSON B, ROTH A. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. *Arch Dis Child* 1982; 57: 364-8.
268. HAMBURGER RB, HELLER S, MELLON M, et al. Current status of the clinical and immunological consequences of a prototype allergic disease prevention program. *Ann Allergy* 1983; 51: 281-90.
269. CHANDRA RK, PURI S, CHEEMA PS. Predictive value of cord blood IgE in the development of atopic disease and role of breast feeding in its prevention. *Clin Allergy* 1985; 15: 517-22.
270. LILJA G, DANNAEUS A, FOUCARD T, GRAFF-LONNEVIG V, JOHANSSON SGO, OMAN H. Effects of maternal diet during late pregnancy and lactation on the development of IgE and egg- and milk-specific IgE and IgG antibodies in infants. *Clin Exp Allergy* 1991; 21: 195-202.
271. LILJA G, DANNAEUS A, FÄLTH-MAGNUSSON K, et al. Immune response of the atopic woman and foetus: effects of high- and lowdose food allergen intake during late pregnancy. *Clin Allergy* 1988; 18: 131-42.
272. HALKEN S, HØST A, JACOBSEN L, JOHANSEN N. Specific IgE antibodies against cow's milk proteins in cord blood. *Allergy* 1993; 48 (suppl 16): 12.

273. GERRARD JW, SHENASSA M. Food allergy: two common types as seen in breast and formula fed babies. *Ann Allergy* 1983; 50: 375-9.
274. KAUFMAN HS. Allergy in the newborn: skin test reactions confirmed by the Prausnitz-Küstner test at birth. *Clin Allergy* 1971; 1: 363-7.
275. FÄLTH-MAGNUSSON K, KJELLMAN NIM. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy - a randomized study. *J Allergy Clin Immunol* 1987; 80: 868-75.
276. FÄLTH-MAGNUSSON K, KJELLMAN N-IM. Allergy prevention by maternal elimination diet during late pregnancy. A 5-year follow-up of a randomized study. *J Allergy Clin Immunol* 1992; 89: 709-13.
277. LILJA G, DANNAEUS A, FOUCARD T, GRAFF-LONNEVIG V, JOHANSSON SGO, OMAN H. Effect of maternal diet during late pregnancy and lactation on the development of atopic diseases in infants up to 18 months of age: *in vivo* results. *Clin Exp Allergy* 1989; 19: 473-9.
278. CHANDRA RK, PURI S, SURAIYA C, CHEEMA PS. Influence of maternal food antigen avoidance during pregnancy and lactation on incidence of atopic eczema in infants. *Clin Allergy* 1986; 16: 563-9.
279. LILJA G. Development of atopy during infancy in relation to food allergen intake. Karolinska Institute, Stockholm, Sweden, 1990. Thesis.
280. LUCAS A, BROOKE OG, MORLEY R, COLE TJ, BAMFORD MF. Early diet of preterm infants and development of allergic or atopic disease: randomized prospective study. *Br Med J* 1990; 300: 837-40.
281. WEAVER LT, LAKER MF, NELSON R, LUCAS A. Milk feeding and changes in intestinal permeability and morphology in the newborn. *J Pediatr Gastroent Nut* 1987; 6: 351-8.
282. HANSON LÅ, AHLSTEDT S, ANDERSSON B, et al. Protective factors in milk and the development of the immune system. *Pediatrics* 1985; 75: 172-6.
283. BRANDTZAEG P, BJERKE K, KETT K, et al. Production and secretion of immunoglobulins in the gastrointestinal tract. *Ann Allergy* 1987; 59: 21-39.
284. TAYLOR B, NORMAN AP, ORGEL HA, STOKES CR, TURNER MW, SOOTHILL JP. Transient IgA deficiency and pathogenesis of infantile atopy. *Lancet* 1973; ii: 111-3.
285. SAVILAHTI E, TAINIO V-M, SALMENPERÄ L, ARIOMAA P, PERHEENTUPA J, SIIMES MA. Low cholostral IgA associated with cow's milk allergy. *Acta Paediatr Scand* 1991; 80: 1207-13.
286. FÄLTH-MAGNUSSON K. Breast milk antibodies to foods in relation to maternal diet, maternal atopy and the development of atopic disease in the baby. *Int Arch Allergy Appl Immunol* 1989; 90: 297-300.
287. ØSTERGAARD PAA. Serum and saliva IgA-levels in infants of non-atopic mothers fed breast milk or cow's milk-based formula. *Acta Paediatr Scand* 1985; 74: 555-9.
288. KRAMER MS. Does breast feeding help protect against atopic disease? Biology, methodology, and a golden jubilee of controversy. *J Pediatr* 1988; 112: 181-90.
289. CHANDRA RK, PURI S, HAMED A. Influence of maternal diet during lactation and use of formula feeds on development of atopic eczema in high risk infants. *Br Med J* 1989; 299: 228-30.
290. ZEIGER RS, HELLER S, MELLON MH, HELSEY JF, HAMBURGER RN, SAMPSON HA. Genetic and environmental factors affecting the development of atopy through age 4 in children of atopic parents: a prospective randomized study of food allergen avoidance. *Pediatr Allergy Immunol* 1992; 3: 110-27.
291. VANDENPLAS Y, DENEYR M, SACRE L, LOEB H. Preliminary data on a field study with a new hypo-allergic formula. *Eur J Pediatr* 1988; 148: 274-7.
292. VANDENPLAS Y, HAUSER B, VAN DEN BORRE C, SACRE L, DAB J. Effect of a whey hydrolysate prophylaxis of atopic disease. *Ann Allergy* 1992; 68: 419-424.
293. BUISSETER PD. Common manifestations of cow's milk allergy in children. *Lancet* 1978; i: 304-6.
294. FIRER MA, HOSKING CS, HILL DJ. Effect of antigen load on development of milk antibodies in infants allergic to milk. *Br Med J* 1981; 283: 693-6.
295. JARRET EEE, STEWART DC. Rat IgE production. I. Effect of dose of antigen on primary and secondary reagenic antibody responses. *Immunology* 1974; 27: 365-81.
296. JARRET EEE, HAIG DM, McDUGALL W, McNUTRY E. Rat IgE production. II. Primary and booster reagenic antibody responses following intradermal or oral immunization. *Immunology* 1976; 39: 671-7.
297. JARRETT EEE. Activation of IgE regulatory mechanism by transmucosal absorption of antigen. *Lancet* 1977; ii: 223-5.
298. LINDFORS A, ENOCKSSON E. Development of atopic disease after early administration of cow milk formula. *Allergy* 1988; 42: 11-6.
299. LINDFORS ATB, DANIELSSON L, ENOCKSSON E, JOHANSSON SGO, WESTIN S. Allergic symptoms up to 4-6 years of age in children given cow milk neonatally. *Allergy* 1992; 47: 207-11.
300. HATTEVIG G, KJELLMAN B, SIGURS N, et al. Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. *Clin Exp. Allergy* 1989; 19: 27-32.
301. SIGURS N, HATTEVIG G, KJELLMANN 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: 735-39.
302. BJÖRKSTÉN B. Does breast feeding prevent the development of allergy? *Immunol Today* 1983; 4: 215-7.
303. JUTO P, BJÖRKSTÉN B. Serum IgE in infants and influence of type of feeding. *Clin Allergy* 1980; 10: 593-600.
304. STRANNEGÅRD I, STRANNEGÅRD IL. T lymphocyte numbers and function in human IgE-mediated allergy. *Immunol Rev* 1978; 41: 149-70.
305. CHANDRA RK, BAKER M. Numerical and functional deficiency of suppressor T cells precedes development of atopic eczema. *Lancet* 1983; ii: 1393-4.
306. JUTO P, MÖLLER C, ENGBERG S, BJÖRKSTÉN B. Influence of type of feeding on lymphocyte function and development of infantile allergy. *Clin Allergy* 1982; 12: 409-16.
307. MELNIK BC, PLEWIG G. Is the origin of atopy linked to deficient conversion of omega 6-fatty acids to prostaglandin E-1? *J Am Acad Derm* 1989; 21: 557-63.
308. BUSINCO L, LOPPI M, MORSE NL, NISINI R, WRIGHT S. Breastmilk from mothers of children with newly developed atopic eczema has low levels of long chain polyunsaturated fatty acids. *J Allergy Clin Immunol* 1993; 91: 1134-9.