

Factors Associated with the Development of Immune Tolerance in Children with Cow's Milk Allergy

Félix Sánchez-Valverde^{a, b} Verónica Etayo^{a, b} Francisco Gil^{a, b} Elena Aznal^{a, b}
Diana Martínez^a Ana Amézqueta^a Mikel Mendizábal^a Arkaitz Galbete^c
Nítida Pastor^d Jon Vanderhoof^e

^aGastroenterology and Paediatric Nutrition Section, Hospital Complex of Navarra, Pamplona, Spain; ^bStudy Group of Nutrition and Paediatric Digestive Diseases of Navarra, GENDINA, Navarra-Biomed, IDISNA, Pamplona, Spain; ^cStatistical Service, Navarra-Biomed, IDISNA, RSSIEC, Navarre Health Service, Pamplona, Spain; ^dMead Johnson Nutrition, Boston, MA, USA; ^eBoston Children's Hospital and Harvard Medical School, Boston, MA, USA

Keywords

Probiotics · Casein hydrolysate · Caesarean delivery

Abstract

Introduction: Cow's milk allergy (CMA) is a common diagnosis in infants, requiring the exclusion of cow's milk until tolerance is recovered. In the present study, we aim to determine which factors are associated with the development of tolerance. **Methods:** Retrospective, observational study of subjects who underwent the same clinical follow-up methodology. We studied 245 cases of CMA (125 IgE-mediated and 120 non-IgE-mediated). The following variables were analysed: age at diagnosis, gender, type of delivery, type of feeding received, feeding during the first months of life, clinical features, and type of feed received as treatment: casein hydrolysates or casein hydrolysates with *Lactobacillus rhamnosus* GG (LGG). **Results:** Factors associated with earlier tolerance were non-IgE-mediated CMA (HR = 2.92; 95% CI: 2.20–3.88) and patients receiving casein hydrolysate with LGG (HR = 1.79; 95% CI: 1.33–2.42). Later tolerance was associated with caesarean delivery (HR = 0.78; 95% CI: 0.58–1.05) and breastfeeding for a period of at

least 3 days (HR = 0.64; 95% CI: 0.44–0.93). The multivariate study shows that the type of formula (HR = 1.61; 95% CI: 1.19–2.18) and the type of CMA (HR = 2.82; 95% CI: 2.12–3.85) have an effect on the recovery time. Casein hydrolysates with LGG reduces the recovery time in IgE-mediated (HR = 1.88; 95% CI: 1.17–3.01) and non-IgE-mediated CMA (HR = 1.46; 95% CI: 0.98–2.17). **Conclusions:** Tolerance acquisition is faster in non-IgE-mediated CMA subjects and in those who received casein hydrolysate with LGG.

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Introduction

Cow's milk allergy (CMA) is one of the most common food allergies among infants. It is estimated to affect 2–3% of children under 5 years of age [1–3]. An IgE- or non-IgE-mediated mechanism or a mixed one can cause this condition [4]. The development period of immune toler-

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ance is variable, with non-IgE-mediated CMA patients showing faster resolution in most cases [5]. In many subjects suffering IgE-mediated CMA, immune tolerance requires more time [6] and recent evidence suggests a worse prognosis and a lower rate of immune tolerance within 4 years from diagnosis [7]. Furthermore, in children with IgE-mediated CMA, the progression to other allergic manifestations later in their life is higher, a process commonly called Allergic (or Atopic) March [8]. Different factors seem to have the potential to influence the immune tolerance acquisition. Among these, the most relevant are the following: family history of allergy (FHA) [9], total serum IgE levels, specific IgE to particular cow's milk protein (CMP) epitopes [7, 10], and the presence of atopic dermatitis [10, 11]. The relevance of gut microbiota in allergic sensitization and food tolerance processes has been highlighted in recent years [12]. Differences in gut microbiota composition and a higher predisposition to trigger Th2 cell-mediated immune responses has been shown in caesarean-born and bottle-fed infants [13–16].

In this study, we aim to investigate the potential factors associated with the acquisition of immune tolerance in IgE- or non-IgE-mediated CMA.

Methods

Study Design and Patients

The study design is a retrospective, observational study on a cohort of children affected by IgE-mediated and non-IgE-mediated CMA. All patients were evaluated at the Gastroenterology and Paediatric Nutrition Unit of the Hospital Complex of Navarra (Pamplona, Spain). The Clinical Research Ethics Committee of the Institution approved the study. Clinical data of all subjects diagnosed with CMA were collected consecutively between January 1996 and January 2014 by expert paediatricians through a consultation of medical records and a recruitment questionnaire.

The inclusion criteria for IgE-mediated cases were: previously healthy patients with clinical features suggestive of CMA [17], positive values of specific IgE antibodies (ELISA Immunocap® system, Phadia AB, Uppsala, Sweden, owned by Thermo Fisher Scientific, Waltham, Massachusetts, USA) to CMP (IgE specific to casein, β -lactoglobulin, and α -lactalbumin, IgE cut-off >0.35 kU/L) in the initial evaluation and confirmed with a positive open oral food challenge (OFC) [18]. The inclusion criteria for the non-IgE-mediated cases were: previously healthy patients with clinical features suggestive of CMA (usually immediate reactions) and no positive values for serum IgE specific to CMP in the initial evaluation and confirmed with a positive OFC. OFCs were performed by the researchers on all patients, except in subjects with a history of anaphylaxis or severe allergic reaction after exposure to CMP or those with levels of specific IgE >2.5 kU/L in the initial determination.

An additional inclusion criterion for both groups was to have received treatment after diagnosis with an extensively hydrolysed casein formula (EHCF; Nutramigen® or Pregestimil®, Mead-

Johnson Nutrition, Evansville, IN, USA) or with an EHCF with *Lactobacillus rhamnosus* GG (LGG; Nutramigen LGG® Mead-Johnson Nutrition, Evansville, IN, USA). Clinicians assigned the treatments. Nutramigen® and Nutramigen LGG® were not commercialized at the same time, thus, Nutramigen LGG® was used from 2005 on and Nutramigen® before that year. We excluded patients without a clear diagnosis of CMA, those with concomitant chronic digestive system disorders, and patients who had already been diagnosed with CMA and had followed previous treatments.

The following data were collected: age at diagnosis, sex, FHA (defined as one or more first-degree family members affected by allergic conditions), type of delivery at birth (vaginal birth or caesarean section), type of feeding received by the newborn during hospital stay (breastfeeding [BF] of at least 3 days or formula), formula feeding during hospital stay (isolated doses of formula during maternity followed by BF exclusively, no supplement with formula discharged on BF exclusively, or discharged on formula feeding), allergic symptoms at diagnosis (urticaria, angio-oedema, perioral erythema, anaphylaxis), digestive symptoms at diagnosis (vomiting, diarrhoea, feed rejection, flat weight curve), respiratory symptoms at diagnosis (spasmodic cough, allergic rhinitis, wheezing), main CMA mechanism (IgE- or non-IgE-mediated), and type of feed received after diagnosis (EHCF with or without LGG). The date of immune tolerance acquisition to CMP was also registered. To show tolerance acquisition, OFCs were performed in all subjects by the researchers following a clinical protocol (at least 6 months after diagnosis and at 1 year of age, treatment well-tolerated and absence of relevant allergic reactions, negativity or significant decrease in levels of IgE-specific antibodies and always <2.5 kU/L). The time of immune tolerance acquisition is defined as the months elapsed between the diagnosis of CMA and the age at which cow's milk was tolerated for the first time (OFC) (with the cut-off date for tolerance recovery set in the study at October 20, 2015).

Statistical Analyses

Mean and standard deviation or median and interquartile range were used to describe the main demographic and clinical characteristics of the subjects for continuous variables and frequencies and percentages for categorical variables. These characteristics are presented for the whole sample and stratified by diagnosis at baseline (IgE-mediated or non-IgE-mediated). The comparison of these characteristics in the diagnostic groups was initially done using the χ^2 test or the t test, depending on the nature of the analysed variables. For the survival analysis, the bivariate association was determined using Kaplan-Meier curves and the log-rank test, which allows determining differences between the curves. We completed the analysis with a multivariate Cox model including the variables of interest in the recovery of CMP tolerance: sex, FHA, type of delivery, type of feeding received during hospital stay, formula feeding during hospital stay, type of CMA, and type of feed received after the diagnosis.

Results

We included 245 cases of CMA (125 IgE-mediated and 120 non-IgE-mediated). Table 1 shows the main demographic and clinical features of the study population. Significant differences were observed regarding the frequen-

Table 1. Clinical and sample identification variables

	Total (n = 245)	IgE (n = 125)	Non-IgE (n = 120)	p (sig.)
Sex, male	54.3	56.8	51.7	0.420
Age at diagnosis, years	4.7±2.7	4.9±2.5	4.5±2.9	0.239
Tolerance, months	20.8±37.2	32.4±49.2	9.0±7.3	<0.001
Caesarean delivery	29.7	39.8	19.0	<0.001
BF ≥3 days, n (%)	214 (87.3%)	122 (97.6%)	92 (76.7%)	<0.001
FFH	63.4	66.7	59.6	0.297
Duration of BF, days	120.6±97.2	134.1±105.3	101.9±81.7	0.015
Family history of allergy	52.3	50.4	54.4	0.559
Allergic symptoms	59.2	78.4	39.2	<0.001
Urticaria	49.2	64.5	33.3	<0.001
Angio-oedema	20.5	29.8	10.8	<0.001
Perioral erythema	21.3	29.8	12.5	<0.001
Anaphylactic shock	0.4	0.8	0.0	0.324
Digestive symptoms	53.1	42.4	64.2	0.001
Vomiting	34.7	40.0	37.3	0.234
Colic	14.3	12.1	16.7	0.202
Diarrhoea	21.7	9.7	34.2	0.001
Respiratory symptoms	6.9	7.2	6.7	0.870
Atopic dermatitis	19.3	20.2	18.3	0.717
EHCF	75.1	81.6	68.3	0.016
EHCF+LGG	24.9	18.4	31.7	0.016

Figures are presented as percentages or mean ± SD unless indicated otherwise. BF, children who were breastfed for at least 3 days; FFH, formula feeding during hospital stay; p (sig.), Student's *t* test (quantitative variables) or χ^2 test (qualitative variables) to compare the IgE-mediated group versus the non-IgE mediated group; EHCF, extensive hydrolyzed casein formula.

cy of caesarean delivery, BF practice, allergic symptoms, and digestive symptoms depending on the mechanism leading to CMA.

The average levels of IgE-specific antibodies in the IgE-mediated group was 5.56 kU/L to casein, 3.47 kU/L to α -lactalbumin, and 5.47 kU/L to β -lactoglobulin. At diagnosis, this group showed high levels of antibodies (>2.5 kU/L): 27.5% to casein, 20% to α -lactalbumin, 30.6% to β -lactoglobulin, and 40.8% to any of them. Eighty percent of the patients who did not achieve tolerance showed high levels of antibodies (>2.5 kU/L) in comparison to patients who acquired tolerance (38.7%), with an OR for high levels of antibodies at diagnosis of 6.33 (2.03–19.69). OFC was performed in almost all non-IgE-mediated patients and in 58.4% of IgE-mediated patients.

Acquisition of tolerance was reached in 78.5% of the subjects, 64% IgE-mediated and 94.2% non-IgE-mediated. Considering the type of formula administered, 78.5% of the total subjects reached tolerance and 93.5% of those who received EHCF with LGG reached it. Immune tolerance acquisition occurred later in the IgE-mediated group in comparison to the non-IgE mediated group (32.4 vs. 9 months on average). Excluding patients that did not achieve toler-

ance, the range of tolerance recovery was from 6 to 141 months. The percentage of mothers who initiated BF was higher in the IgE-mediated group (97.6 vs. 76.7%). The comparative duration of BF between these two groups was longer in the IgE-mediated group (134.1 vs. 101.9 days).

Both groups were fed more frequently with EHCF than with EHCF+LGG, with differences being higher in the IgE-mediated group (81.6 vs. 18.4%) than in the non-IgE-mediated group (68.3 vs. 31.7%). The results of the univariate analysis in Table 2 shows that the factors associated with the time acquisition of immune tolerance are type of CMA, BF, and type of feeding received following the diagnosis.

Survival curves consider as a censored case the patient who has not achieved immune tolerance at the end of the follow-up period. The curves in Figure 1 show the variables found to be associated with immune tolerance acquisition over time (months); rapid tolerance acquisition was seen in a high proportion of patients. At 10–15 months, the curves separate, with an estimated 10% in some groups of patients who do not recover tolerance.

Multivariate analysis considering the total sample size (Table 3) showed that the variable type of CMA and type of feeding received after diagnosis remain in the model,

Table 2. Univariate Cox regression model for tolerance recovery

Variable	HR	95% CI	<i>p</i> (sig.)
Sex			
Male	Ref.		
Female	1.06	0.82–1.38	0.666
Type of CMA			
IgE-mediated	Ref.		
Non-IgE-mediated	2.92	2.20–3.88	<0.001
Type of feeding following diagnosis			
EHCF	Ref.		
EHCF+LGG	1.79	1.3–2.42	<0.001
Type of delivery			
Vaginal	Ref.		
Caesarean	0.78	0.58–1.05	0.095
BF (≥3 days)			
No	Ref.		
Yes	0.64	0.44–0.93	0.021
FFH			
Yes	Ref.		
No	1.05	0.78–1.41	0.771
FHA			
No	Ref.		
Yes	1.06	0.81–1.40	0.643

CMA, cow's milk allergy; EHCF, extensive hydrolyzed casein formula; BF, breastfeeding for a period of at least 3 days; FFH, isolated formula feeding during hospital stay followed by BF exclusively; FHA, family history of allergy; HR, hazard ratio (values >1 means quicker tolerance acquisition).

while variables such as type of delivery or BF loose statistical significance. In all subjects with CMA, the recovery rate increased by 61% when fed with EHCF+LGG; in non-IgE-mediated CMA, the rate of tolerance recovery increased threefold.

The stratified analysis of the IgE-mediated and non-IgE-mediated groups (Table 4) revealed that the type of hypoallergenic formula given could be linked to the recovery of tolerance in the IgE-mediated group (HR = 1.88, 95% CI: 1.17–3.01; *p* = 0.002; adjusted by IgE antibodies levels), whereas in the non-IgE-mediated group it is close to significance. On the other hand, the type of delivery and BF clearly loose significance in both groups.

Discussion

The results of this study confirm that the immune mechanism (IgE- or non-IgE-mediated) drives relevant differences in terms of clinical features and the progres-

sion of CMA, as reported elsewhere [5, 19]. We ratify [20–22] that immune tolerance acquisition occurs faster in non-IgE-mediated CMA in comparison to IgE-mediated.

The relationship between caesarean section and IgE-mediated CMA has been previously described [15, 23, 24]. It is generally accepted that this is due to an alteration in intestinal microbiota during caesarean deliveries that persists through the first months of life [13, 14, 16, 20]. We found a clear association between caesarean deliveries and IgE-mediated CMA at the time of diagnosis, although the relationship to the Allergic March could not be demonstrated later on [15].

We believe that the intake of isolated cow's milk formula during the first hours of life, together with the effect caesarean sections have on intestinal bifidogenic flora, may be associated with IgE-mediated CMA, the former being a predisposing factor and the latter a triggering one [25]. Our results show that caesarean sections could be associated with delayed acquisition of tolerance in patients affected with CMA. The observed loss of strength in the multivariate analysis may be due to the relationship of caesarean delivery with IgE-mediated cases, which are more likely to present prolonged tolerance acquisition times.

It is widely accepted that the time at which BF is initiated, as well as its duration, has an effect on the protection against atopy in general [26] and particularly against CMA [21]. In our study, initial BF is associated with later tolerance acquisition, contrary to what has been reported elsewhere. This could be explained by the fact that an IgE-mediated condition is characterized by a process of sensitization against CMP in the newborn produced by low-grade sensitization (isolated doses of CMP), risk factors such as caesarean section and continuation of BF. However, in non-IgE-mediated cases, contact with artificial formulae occurs sooner and consequently BF is abandoned earlier because of the perceived gastrointestinal symptoms [27]. Furthermore, considering the loss of significance observed in the multivariate analysis, the effect may be related to the strength association of BF and IgE-mediated CMA, which are more likely to present prolonged tolerance acquisition times.

We were not able to confirm the influence of allergic disease history of first-degree relatives on tolerance recovery. The relationship between CMA and family atopy profile has been documented in other studies [9, 28]. In a previous study [15], we show that FHA is not related to the development of IgE- or non-IgE-mediated CMA, although we did find an association with the development of Allergic March (other allergic diseases: asthma, allergic

Fig. 1. Tolerance recovery (log-rank) considering initial diagnosis, type of feeding after the diagnosis, type of feeding during hospital stay (breastfeeding for a period of at least 3 days) and type of delivery. EHCF, extensively hydrolysed casein formula; LGG, *Lactobacillus rhamnosus* GG.

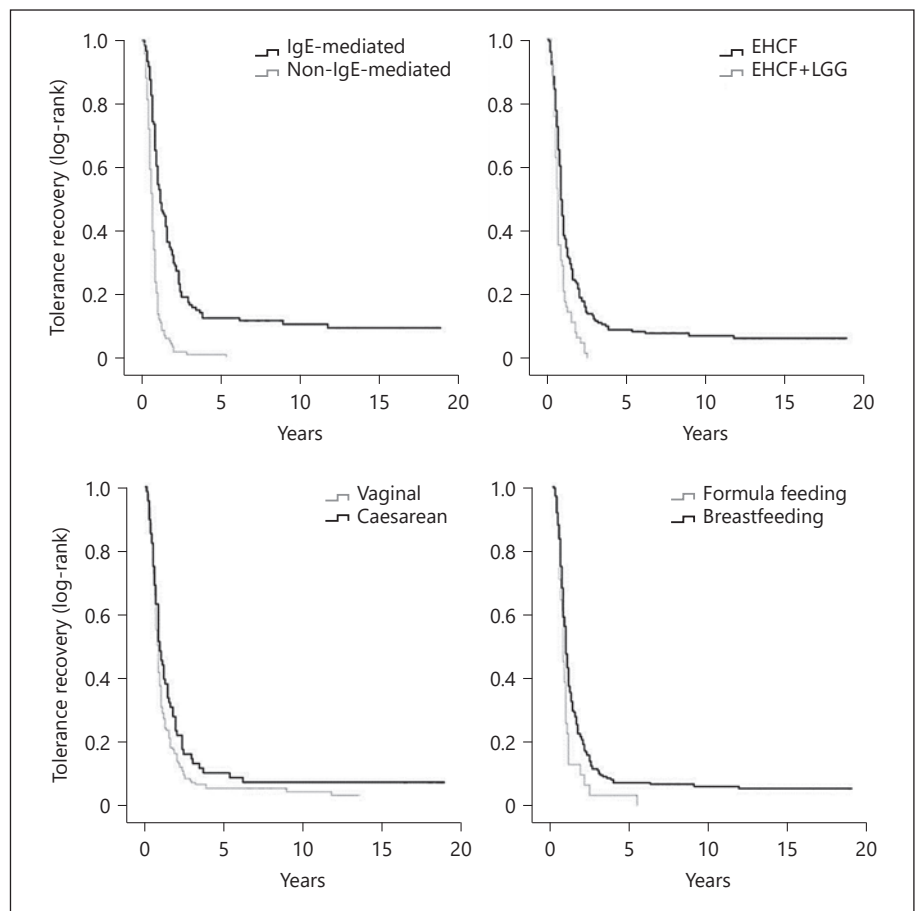


Table 3. Multivariate Cox regression model for tolerance recovery

Variable	Category	HR	95% CI	<i>p</i> (sig.)
Type of CMA	IgE-mediated	Ref.		
	Non-IgE-mediated	2.82	2.12–3.75	<0.001
Type of feeding after diagnosis	EHCF	Ref.		
	EHCF+LGG	1.61	1.19–2.18	0.002

CMA, cow's milk allergy; HR, hazard ratio (values >1 mean quicker tolerance acquisition); EHCF, extensive hydrolyzed casein formula.

Table 4. Stratified Cox regression model for tolerance recovery

Group	Category	HR	95% CI	<i>p</i> (sig.)
IgE-mediated	EHCF+LGG	1.78	1.11–2.86	0.001
	Specific IgE to CMP >2.5 KU/L	0.50	0.33–0.74	0.016
Non-IgE-mediated	EHCF+LGG	1.46	0.98–2.17	0.069

HR, hazard ratio (values >1 means quicker tolerance acquisition); EHCF, extensive hydrolyzed casein formula; CMP, cow's milk protein.

rhinitis, atopic dermatitis or other food allergies). Tolerance acquisition could act as an intermediate link throughout the course of this disease. The absence of a relationship could be due to the high proportion of relatives with allergic diseases (in both groups, almost 50% had one first-degree family member with an allergic condition). We did not observe differences in the proportion of FHA between the two groups nor in our general population. In developed communities like ours, the prevalence of self-referred allergic diseases is probably high, and a single parent or sibling with allergy may not be a specific marker of family risk.

A relevant factor that can affect the time to immune tolerance acquisition in CMA is the formula used after the diagnosis. Children treated with EHCF+LGG show faster acquisition of tolerance. This association remains significant when adjusted by the immune mechanism. Furthermore, when we carried out a stratified analysis, the significance is maintained for the IgE-mediated group and is close to significant for the non-IgE-mediated group ($p = 0.069$). Our observations that children given LGG in formula develop tolerance at an earlier age supports the concept that the microbiota in the gastrointestinal tract of the infant can significantly influence the immune priming and the regulation of immune tolerance.

Our results on earlier recovery of tolerance in infants treated with EHCF+LGG are consistent with data published by Berni Canani et al. [29, 30]. Although our data were more robust in IgE-mediated CMA, differences were observed close to significance in non-IgE-mediated cases, suggesting that modulation of the microbiota may also be beneficial for this group. Further studies are needed to better understand the mechanisms involved in these observations and to determine whether other factors are important.

A limitation of this study is the design of the methodology. The frequency of the disease in our context resulted in an extended recruitment period to include a sufficient sample size. After a period of almost 20 years, a bias of loss of information would be expected. However, this study is part of a big cohort of CMA patients whose control and follow-up are carried out via computerized registries of the clinical histories and loss of data was low. Furthermore, certain variables such as the socio-economic status of the mothers, rural or urban likelihood, use of antibiotics, concomitant BF or use of other probiotics that could have an influence on gut microbiota were not studied. Missing these variables could make the current results subject to residual confounding.

We conclude that non-IgE-mediated patients recovered tolerance faster than IgE-mediated ones. Moreover, patients fed with EHCF+LGG recovered tolerance faster than those fed with EHCF without LGG. A faster recovery in IgE-mediated patients fed with EHCF+LGG was observed in the stratified analysis and the same trend in non-IgE-mediated patients (although the latter did not reach statistical significance).

Statement of Ethics

This study was approved by the Ethics Committee of Human Investigation of our institution (following the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments).

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The authors declare that they have no conflict of interest. This research was developed by the Paediatric Nutrition and Digestive Research Group of Navarre. It has received partial aid from the Government of Navarre and from Mead-Johnson Nutrition, Evansville, IN, USA.

Author Contributions

Dr. Sánchez-Valverde conceptualized and designed the study, carried out the initial analyses, drafted the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Dr. Etayo reviewed and revised the manuscript and approved the final manuscript as submitted.

Dr. Gil conceptualized and designed the study, coordinated and supervised data collection, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Dr. Aznal reviewed and revised the manuscript and approved the final manuscript as submitted.

Dr. Martínez collaborated with the acquisition of data, reviewed and revised the manuscript and approved the final manuscript as submitted.

Dr. Amézqueta collaborated with the acquisition of data, reviewed and revised the manuscript and approved the final manuscript as submitted.

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