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Faecal Microbiota and Short-Chain Fatty Acid Levels in Faeces from Infants with Cow's Milk Protein Allergy

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Key Words

Allergy and immunology, child · Fatty acids · Gut microbiota · Intestine, infants · Intestinal microflora · Microbiology · Milk hypersensitivity · Short-chain fatty acids

Abstract

Background: The present study was designed to compare the faecal microbiota and concentrations of faecal short-chain fatty acid and ammonia between healthy and cow's milk protein allergic (CMPA) infants. ***Methods:*** The population comprised 92 infants aged 2–12 months who were non-allergic (n = 46) or diagnosed as having CMPA (n = 46). Faecal samples were analyzed by fluorescent in situ hybridization and flow cytometry, using a panel of 10 rRNA targeted group- and species-specific oligonucleotide probes. Acetic, propionic, butyric, isocaproic and branched-chain short fatty acids (BCSFA) were measured by gas-liquid chromatography, lactate by enzymatic reaction, and pH and ammonia levels were determined. ***Results:*** CMPA infant faeces had significantly higher proportions of the *Clostridium coccoides* group and *Atopobium* cluster and a higher sum of the proportions of the different bacterial groups in comparison to healthy infant faeces. Faecal pH and ammonia did not significantly differ between CMPA and healthy infants. Faeces concentrations and percentages of butyric acid and BCSFA were higher in CMPA infants than in healthy infants. ***Conclusions:*** The findings clearly set a link between a dysbiosis in gut microbiota composition and the pathogenesis of CMPA. No single species or genus appeared to play an essential role, but dysbiosis led to biomarkers of CMPA among bacterial fermentation products.

Introduction

Cow's milk protein allergy (CMPA) is the most common food allergy during early childhood, with an incidence of 2–3% in the first year of life [1]. It has been hypothesized that the gut microbiota might be involved in the aetiology of atopic disease [2], but no specific harmful or protective microbes have yet been identified. Some investigations, based exclusively on traditional bacteriological culture techniques, reported major differences in gut

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microbiota between healthy and allergic infants and postulated a possible association between allergy and an altered microbiota pattern [3–5]. However, no research on microbiota has been published in infants diagnosed as having CMPA.

A large number of human gut bacteria cannot be cultured, and it has been reported that 60–80% of gut bacteria have not yet been characterized [6]. Gut microbiota can be investigated either by traditional bacteriological culture techniques or by molecular techniques or by exploration of microbiota-associated metabolic characteristics. New techniques based on bacterial RNA and DNA have been developed for investigating, identifying and quantifying the intestinal microbiota. Fluorescent in situ hybridization (FISH) combined with flow cytometry (FC) is a high-throughput method based on 16S rRNA probe hybridization that reliably characterizes the composition of faecal microbiota [7]. A case-control study found no differences in concentrations of specific genera between healthy infants and infants suffering from atopic dermatitis [8]. A prospective birth cohort study observed a tendency for atopic infants to have fewer bifidobacteria and more clostridia [9], whereas another study reported fewer clostridia in children with eczema than in healthy controls [10].

The metabolic activities of gut bacteria play an important role in human health and disease. The end products of bacterial fermentation are diet dependent. Bacterial fatty acid profiles differ between faecal samples collected before and after weaning [11]. Concentrations of short-chain fatty acids (SCFA) in faecal samples have also been found to differ between sensitized and healthy infants [12, 13], but we have been able to trace a report on infants with CMPA.

With this background, this study was designed to compare faecal microbiota (by using FISH-FC) and levels of faecal SCFA and ammonia between healthy and CMPA infants in a Spanish population.

Subjects and Methods

Patient Enrolment and CMPA Diagnoses

During a 17-month period (May 2005 to October 2006) at the San Cecilio University Hospital (Granada, Spain), all consecutive infants referred to the pediatric department for suspicion of allergy and diagnosed as having CMPA were enrolled in the study. Three tests were performed to establish the diagnosis: (1) skin prick test with whole cow's milk extract, α-lactalbumin, β-lactoglobulin and casein (Laboratorios Leti, Barcelona, Spain), using histamine dihydrochloride (10 mg/ml) as positive control and saline solution as negative control; reactions were read at 15 min; a net wheal diameter 3 mm larger than that produced by the negative control was considered positive; (2) analysis of serum samples from all infants for specific IgE antibodies to cow's milk, α-lactalbumin, β-lactoglobulin and casein, by using a Cap system (ImmunoCap 250; Phadia, Uppsala, Sweden), considering ≥0.35 kU/l a positive result, and (3) a double-blind placebo control cow's milk challenge. The challenge was considered to be positive when there were skin (urticaria, angioedema, or erythematous rash), gastrointestinal (vomiting or diarrhoea), respiratory (rhino-conjunctivitis or bronchospasms) or generalized (anaphylactic shock) manifestations in the 2 h after the intake of the food.

The challenge test was considered contraindicated in cases of anaphylactic shock and/or gottal oedema, and nonindicated in patients who met all the following criteria: (1) urticaria and/or angioedema; (2) appearance of symptoms in the first 60 min after intake, and (3) positive skin tests (>3 mm) and specific IgE >3 kU/l to any of the CMP.

The patient was considered to show CMPA IgE-mediated when the following criteria were met: (1) a clear history of immediate hypersensitivity to CMP; (2) positive skin test, specific IgE to any of the CMP, or both, and (3) positive cow's milk challenge test.

The study population comprised 46 CMPA IgE-mediated infants (26 girls and 20 boys), aged 6 months on average (range = 2–12 months), all residents in the urban area of Granada, Spain.

During the study enrolment period, age- and sex-matched controls (26 girls and 20 boys), all residents in Granada, were recruited from among healthy infants coming to the paediatric department for periodic check-up and who showed no allergic symptoms. These children had been exclusively breast fed until introduced to milk formula at the same age as the paired CMPA infants. For ethical reasons, it was not possible to carry out any test in the control group to rule out the possibility of asymptomatic CMPA. Neither CMPA infants nor healthy controls had received antibiotics for a period of at least 3 weeks. Table 1 summarizes the information for CMPA and control infants about the age

Table 1. Age at diagnosis, mode of delivery, duration of breast-feeding, age of weaning and diet at time of sampling in CMPA and control infants

Characteristics	Cases (n = 46)	Controls (n = 46)
Mode of delivery		
Vaginal	42	41
Cesarean	4	5
Duration of breast-feeding		
<3 months	21	17
>3 months	25	29
Mean age of weaning, weeks	17.0 (8–24)	16.7 (6–42)
Diet at time of sampling		
Artificial feeding	20	18
Mixed breast- and artificial feeding	21	19
Breast-feeding	5	9

Figures in parentheses are ranges.

Table 2. Panel of probes used for in situ assessment of microbiota composition of faeces of infants

Probes	Sequence from 5′ to 3′ end	Targeted groups	Reference
Bif 164	CATCCGGCATTACCACCC	*Bifidobacterium* genus	[20]
Bac 303	CCAATGTGGGGGACCTT	*Bacteroides* group	[21]
Enter 1432	CTTTTGCAACCCACT	Enterobacteria	[22]
Strc 493	GTTAGCCGTCCCTTTCTGG	*Streptococcus* group	[19]
Lab 158	GGTATTAGCAYCTGTTTCCA	*Lactobacillus* group	[23]
Ato 291	GGTCGGTCTCTCAACCC	*Atopobium* cluster	[18]
Erec 482	GCTTCTTAGTCARGTACCG	*Clostridium coccoides* group	[19]
Clep 866	GGTGGATWACTTATTGTG	*Clostridium leptum* group	[24]
Cpef 191	GCTCCTTTGGTTGAATGATG	*Clostridium perfringens* sps.	[17]
Cdif 198	TCCATCCTGTACTGGCTCACC	*Clostridium difficile* sps.	[17]

of diagnosis, mode of delivery, duration of breast-feeding, age of weaning and diet at time of sampling.

The study was approved by the ethics committee of the San Cecilio University Hospital in Granada, Spain. The parents were informed verbally and in writing about the nature and requirements of the study, and their written informed consent was obtained.

Faecal Samples and Cell Fixation

Faecal samples were collected at the hospital from all participating infants into sterile plastic tubes by 2 researchers (J.M. and O.C.T.-C.) and immediately placed into anaerobic jars (Anaerogen™; Oxoid, Hampshire, UK). The samples were sent to the laboratory and processed within 2 h of their collection. The cell fixation process was performed as previously described [14]. The faeces were homogenized by mechanical kneading for 3 min and an aliquot of 1 g (wet weight) was added to 9 ml of anaerobic phosphate-buffered saline (PBS). The suspension was mixed to complete homogeneity in a 50-ml stoppered sterile glass jar fitted with a magnetic bar and an aliquot of 0.2 ml of the suspension was added to 0.6 ml of 4% para-formaldehyde (PFA) in PBS. After 1 night at 4°C, the PFA-fixed suspensions were stored at –70°C and shipped on dry ice for analysis at a single location (INRA, Jouy en Josas, France).

FISH of Bacterial Cells

The FISH methodology used in this study is described elsewhere [7, 14]. In brief, 400 μl of fixed suspension was mixed with 600 μl of PBS. Before hybridization, the cells were always pelleted at 8,000 *g* for 3 min in a microcentrifuge tube and resuspended in a volume of 1 ml. After washing in Tris-EDTA buffer (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0), the pellets were suspended in Tris-EDTA buffer containing 1 mg/ml of lysozyme (Serva, Heidelberg, Germany) and incubated for 10 min at room temperature. The cells were then washed in PBS to remove lysozyme and equilibrated in hybridization solution (900 mM NaCl, 20 mM Tris-HCl, pH 8.0, 0.01% sodium dodecyl sulfate, pH 7.2, 30% formamide). A 50-μl aliquot of this suspension was used for FISH with control and species-specific probes. Hybridization was performed in a 96-well microtiter plate overnight at 35°C in the hybridization solution containing 4 ng/μl (final concentration) of the appropriate labelled probes. Following hybridization, 150 μl of hybridization solution was added to each well and cells were pelleted at 4,000 *g* for 15 min. Non-specific binding of the probe was removed by incubating the bacterial cells at 37°C for 20 min in washing solution (64 mM NaCl, 20 mM Tris HCl, pH 8.0, 5 mM EDTA, pH 8.0, 0.01 sodium dodecyl sulphate, pH 7.2). The cells were finally pelleted at 4,000 *g* for 15 min and resuspended in PBS. An aliquot of 100 μl was added to 0.4 ml of FACS Flow for FC detection.

The EUB 338 probe, targeting a fully conserved region within the domain bacteria [16], was used as positive control of hybridization and the NON 338 probe [17] as a negative control. The oligonucleotide probes were covalently linked at their 5′ end with indodicarbocyanine (Cy5) (Thermo Electrom, Ulm, Germany). A panel of 10 group- and species-specific probes covalently linked with Cy5 and their 5′ end was used to assess the microbiota composition [18, 24] (table 2). Enumeration of the different bacterial groups or species was performed by FISH-FC by combining a specific Cy5-labelled probe and EUB 338 FITC-labelled probe in the same tube.

Data acquisition was performed with a FACS Calibur flow cytometer (Becton Dickinson, Erembodegen-Aalst, Belgium) equipped with an air-cooled argon ion laser providing 15 mW at 488 nm combined with a 635-nm red-diode laser, as described previously [7, 15]. Analyses were performed as described elsewhere [25], constructing density plots and delineating regions to encompass double-labelled bacteria.

Determination of Faecal SCFA

Aliquots of fresh faeces were weighed and freeze-dried for calculation of faecal water [11] and direct measurement of pH with a microelectrode MI-410 (Microelectrodes Inc., New Hampshire, USA). For SCFA analysis, 50 mg (+1 mg) sample was weighed into a 10-ml HDPE tapered tube (Nalgene), to wich 0.8 ml deionized water, 100 μl orthophosphoric acid and 100 μl internal standard (2-ethyl-buteric acid, Acros Organics, Geel, Belgium) were added. The contents were mixed by vortex and extracted in triplicate with 3 ml diethylether. A quantity of the pooled extract (containing the acidified SCFA) was transferred into a 2-ml glass vial and loaded onto a ThermoQuest AS 2000 autosampler set to inject 1 μl (inlet temp. 230°C) driven by Chrom-Card Software. Separa-

Table 3. Composition of faecal microbiota of CMPA and healthy infants

	Cases (n = 46)	Controls (n = 46)
Bifidobacterium genus	14.3 (0.0–85.8)	3.0 (0.0–70.6)
C. coccoides group	19.3 (0.0–54.9)*	1.1 (0.0–42.3)
C. leptum group	0.0 (0.0–39.6)	0.0 (0.0–24.3)
Atopobium cluster	0.6 (0.0–45.3)*	0.0 (0.0–11.1)
Bacteroides group	15.7 (0.0–56.0)	13.0 (0.0–82.6)
Enterobacteria group	1.1 (0.0–8.8)	1.0 (0.0–35.9)
Lactobacillus group	0.1 (0.0–2.5)	0.24 (0.0 42.9)
Streptococcus group	0.2 (0.0–11.5)	0.37 (0.0–15.8)
C. perfringens + *C. difficile*	0.4 (0.0–8.9)	0.3 (0.0–16.3)
Sum of the proportions	76.6 (39.6–104.2)*	55.9 (8.6–133.9)

Enumeration of the different bacterial groups or species was quantified by FISH-FC by combining a specific Cy5-labelled probe (10 group- and species-specific probes) and EUB 338 FITC-labelled probe in the same tube. The EUB 338 probe, targeting a fully conserved region within the domain bacteria, was used as positive control of hybridization, and the NON 338 probe was used as negative control. Values are expressed as median proportions and ranges of the bacterial groups. * $p < 0.01$.

tion was afforded on a Trace 2000 Series GC fitted with a ZB WAX Polyethylene column (15 m × 0.53 mm ID × 1.0 m FT Zebron; Phenomenex, Macclesfield, UK) with nitrogen carrier (constant flow of 12 ml/min) and programmed temperature (80–210°C at 15°C/min). Detection was achieved using a flame ionization detector. Peaks were identified using a mixed external standard and quantified by peak height/internal standard ratio.

Lactate Analysis

Prior to enzymatic determination, 30 mg + 0.1 mg of freeze-dried sample was weighed into a 2-ml Safe-Lock microcentrifuge tube (Fisher Scientific, Loughborough, UK), and solubilized by addition of 2 ml deionized water, mixing by vortex and incubating for 15 min at 60°C in a preheated water bath. Protein was removed via a 2-step clarification with Carrez reagents (I) potassium hexacyanoferrate and (II) trihydrate (K_4 [Fe $(CN)_6$] × $3H_2O$ 15 g/100 ml) and (II) zinc sulphate heptahydrate ($ZnSO_4$ × 7 $3H_2O$ 30 g/100 ml, BIOQUANT; Merck, Darmstadt, Germany). Total lactate was determined enzymatically using a D- and L-lactate detection kit (Roche, Boehringer Mannheim/R-Bioopharm, Darmstadt, Germany).

Ammonia

Ammonia was measured with an ammonia selective electrode (ion-specific meter, Hanna, Padua, Italy), after the addition of ionic strength adjustor to raise the pH above 12. This converted the NH_4 ions into gaseous NH_3. The electrode measured the released gas, and concentrations of the samples were determined for comparison with series of standard solutions with known concentrations [26].

Statistical Analysis

The distribution of variables was obtained by calculating the asymmetry and kurtosis and using the Wilcoxon test of normality. Because no variables were normally distributed, even after logarithmic transformation, data were expressed as medians and ranges (minimum–maximum). Nonparametric tests were used to compare data from allergic and control infants. The Mann-Whitney U- test was used for comparisons between unpaired groups (allergy vs. control) and the χ^2 test for categorical determinations and proportions. Correlations were assessed by calculating the Spearman correlation coefficients. SSPS 15.1 for Windows software (SSPS Inc., Chicago, Ill., USA) was used for all data analyses, considering $p < 0.05$ to be significant.

Results

Bacterial Counts (FISH-FC)

The faecal proportions of the different bacterial groups analyzed in this study are given in table 3. CMPA infant faeces had significantly larger proportions of the *Clostridium coccoides* group and *Atopobium* cluster and a higher sum of the proportions of the different bacterial groups.

pH, Ammonia, SCFA and Lactate

No significant difference was found between CMPA and healthy infants in faecal pH [5.8 (4.5–7.1) vs. 6.0 (4.7–7.9)], ammonia [40.6 (0.85–124.1) vs. 27.4 (13.6–70.6) mmol/kg faeces] and water percentage [77.8 (70.7–86.1) vs. 77.2 (51.7–89.5)].

Faecal concentrations of butyric acid and branched-chain short fatty acids (BCSFA) were higher in CMPA versus healthy infants (fig. 1). The percentages of butyric acid [6.7 (0.0–30.2) vs. 1.8 (0.0–37.1); $p < 0.05$] and BCSFA [3.1 (0.0–14.7) vs. 1.2 (0.0–7.4); $p < 0.01$] were also higher in faeces from CMPA versus healthy infants. No significant difference was found between CMPA and healthy infants and faecal lactate (fig. 1). Lactate and acetate were present in all faecal samples; propionic acid was absent in only 3 samples of the control group; butyric acid was absent in 12 control and 3 CMPA samples, and isocaproic acid in 24 control and 25 CMPA samples.

Relationship between Bacterial Group and Faecal SCFA

Table 4 shows the significant correlations between bacterial percentages and levels or percentages of SCFA, and levels of lactate.

In the CMPA infants, percentages of both groups of dominant faecal *Clostridium* were positively correlated with the level and percentage of butyric acid and the *C.*

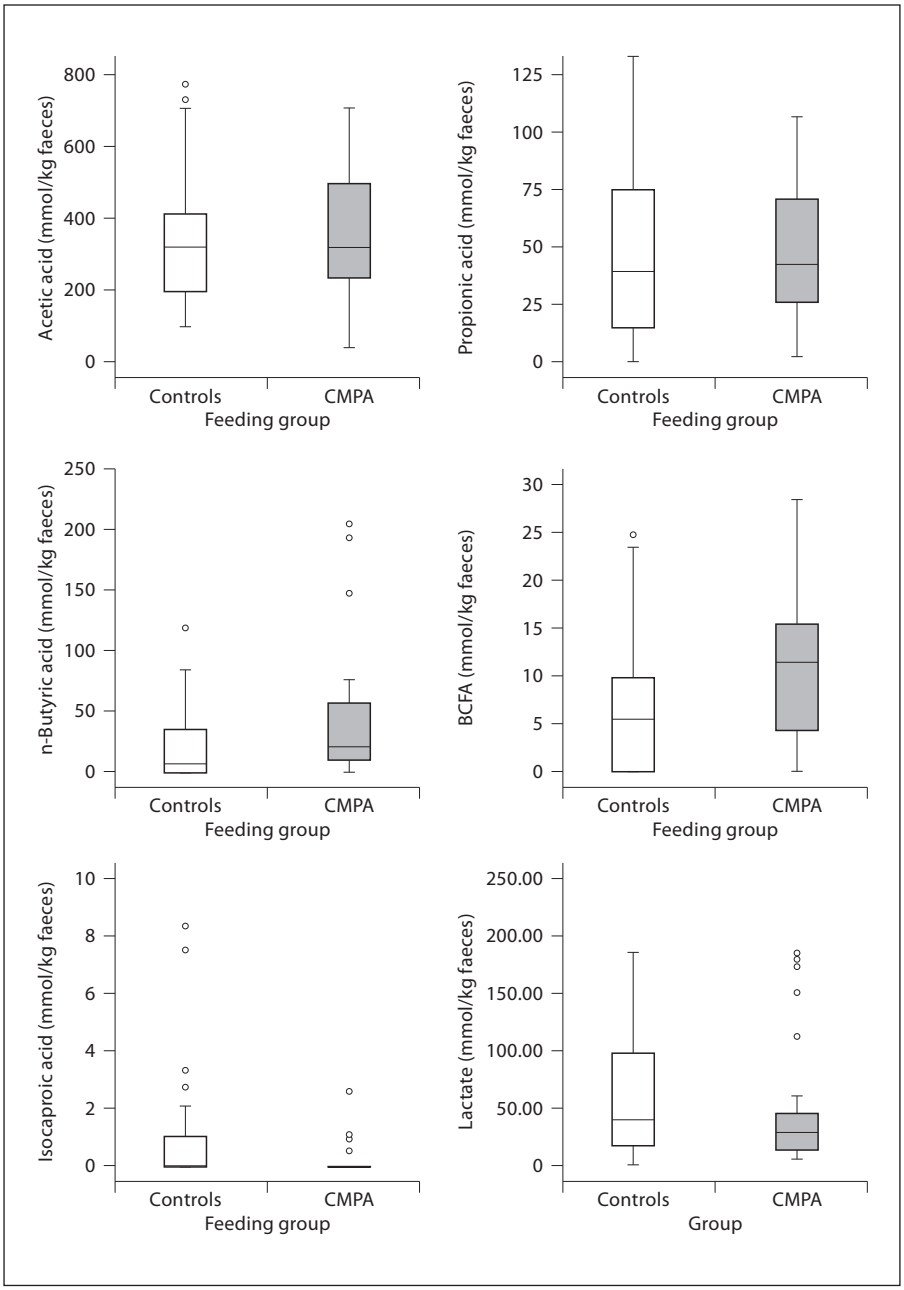

Fig. 1. Concentrations of acetic, propionic, butyric, BCSFA, isocaproic and lactate in faecal samples from healthy (controls) and CMPA (cases) infants. Values are expressed as medians and ranges.

coccoides percentage was associated with BCSFA levels. *Bacteroides* percentage was also positively correlated with the levels and percentage of propionic acid. The genus *Bifidobacterium* was negatively correlated with the levels and percentages of BCSFA and isocaproic acid in these children.

In the healthy infants, *C. coccoides* and *Bacteroides* percentages were correlated with the level and percentage of propionic acid, *Streptococcus* and *Clostridium perfringens* + *Clostridium difficile* percentages were correlated with the level and percentage of butyric acid, and *C. perfringens* + *C. difficile* percentages with the percentage and level of isocaproic acid.

The lactate levels were positively correlated with the *Bifidobacterium* genus and negatively with *C. coccoides*, reaching significance only in CMPA infants.

Table 4. Most relevant significant correlations between faecal bacterial percentages and concentrations and percentages of short-chain fatty acids

Correlations	Cases (n = 46)		Controls (n = 46)	
	mmol/kg faeces	%	mmol/kg faeces	%
Propionic acid				
C. coccoides group	0.168	0.188	0.580**	0.463**
Bacteroides group	0.573**	0.638**	0.542**	0.593**
Butyric acid				
C. coccoides group	0.583**	0.582**	0.363*	0.349
C. leptum group	0.559**	0.505**	0.338	0.387*
Enterobacteria group	–0.313	–0.398*	0.360*	0.319
Streptococcus group	0.395*	0.364	0.387*	0.382*
C. perfringens + *C. difficile*	0.470*	0.613**	0.371*	0.394*
Branched-chain fatty acids				
Bifidobacterium genus	–0.482**	–0.432**	0.083	0.051
C. coccoides group	0.516**	0.425**	0.212	0.107
Isocaproic acid				
Bifidobacterium genus	–0.449**	–0.459*	0.091	0.069
C. perfringens + *C. difficile*	–0.203	0.194	0.353*	0.378*

* $p < 0.05$; ** $p < 0.01$.

Discussion

The most important findings of the present study were that the *C. coccoides* group and *Atopobium* cluster were both significantly more represented in the faeces of CMPA infants. No differences in *Bifidobacterium* genus or *Lactobacillu*s and *Bacteroides* groups were found between CMPA and healthy infants, but the faecal levels and percentages of butyric acid and BCSFA were higher in the CMPA infants.

The prevalence of colonization by clostridia in CMPA infants was previously described in culture-based studies, which associated high clostridia counts with clinical manifestations of allergy and specific IgE antibodies to food and for inhalant allergens [27]. However, in contrast to the present findings, the prevalence of colonization by bifidobacteria was reported to be consistently lower in infants who developed allergy than in those who did not [4, 27, 28], whereas for lactobacilli some reports less often found colonization in allergic children [28] and some others did not find significant changes [4, 27].

Our findings are in partial agreement with recent molecular studies. Kalliomäki et al. [9] found a reduced *Bifidobacterium*:*Clostridium* ratio to be characteristic of the gut microbiota in high-risk 3-week-old neonates who later developed atopic sensitization at 12 months. In contrast, Watanabe et al. [29] and Mah et al. [10] reported a lower clostridia count in cultured faecal samples from infants with eczema versus controls. Kirjavainen et al. [8] observed no differences in concentrations of specific genera between healthy infants and infants suffering from atopic dermatitis but found that higher bacteroides counts and lower bifidobacteria counts were associated with severer manifestations or atopic dermatitis. They also reported that exclusively breast-fed infants who were later diagnosed as having CMPA had higher counts of bacteroides during breast-feeding in comparison to atopic infants who did not develop CMPA. Mah et al. [10] also found that infants with eczema had lower bifidobacteria counts and higher lactic acid bacteria counts versus healthy controls. In contrast, Murray et al. [30] observed no difference in the prevalence of lactic acid bacteria and bifidobacteria in the faeces of sensitized children with history of recurrent wheeze in comparison to nonsensitized, nonwheezy controls; they suggested that the gut microbiota may play different roles in children with distinct allergy disorders, given the diversity of allergy disease phenotypes.

Studies of microbiota-associated characteristics [12, 13] showed that allergic children had higher levels of isocaproic acid and lower levels of the other SCFA in comparison with nonallergic children. In contrast, the pres-

ent study found a higher concentration and percentage of butyric acid in CMPA versus nonallergic infants.

Isocaproic acid is thought to indicate the presence of *C. difficile* in the gut [31]. It is hypothesized that clostridia have immunoregulatory properties [32] and may predispose to allergy development. Proposals regarding the cellular and molecular mechanisms that underlie these phenomena include changes in the fine balancing of Th1, Th2 and T regulatory cell responses, due to suppressed Th1 function [33]. The abrogation of oral tolerance and inflammatory response of intestinal mucosa have also been implicated; the intestinal permeability is increased in inflamed states, allowing the passage of dietary antigens into de lamina propria of the gut [29, 33].

Higher concentrations of the butyric acid can increase the permeability of intestinal mucosa. Lin et al. [34] demonstrated that high levels of butyric acid induced mucosal injury in newborn rats, which was associated with a marked downregulation of intestinal trefoil factor (important for gastrointestinal mucosal defence and healing) gene expression. They speculated that a major downregulation of the intestinal trefoil factor gene expression by SCFA in vivo may play a role in the pathogenesis of necrotizing enterocolitis in premature infants likely to develop elevated intraluminal SCFA levels. Butel et al. [35] also demonstrated that a reduction in luminal butyric acid concentration resulting from bifidobacteria colonization correlates with attenuated intestinal mucosal injury. In our CMPA infants, there was a positive correlation between *Clostridium* percentages and the faecal concentration of butyric acid, and a negative correlation between the *Bifidobacterium* genus and isocaproic acid levels. Our data present further evidence of a disturbed gut microbiota in CMPA infants.

Bifidobacteria and lactobacilli are considered beneficial to the host and can restrain harmful microbiota. Our data indicate that counts of these micro-organisms are not significantly altered in CMPA infants. Recent observational studies in atopic infants suggested that neither bifidobacteria nor lactobacilli play a special role in preventing or promoting the initial development of atopy [2]. Ouwehand et al. [5] reported that allergic infants harboured an adult *Bifidobacterium* microbiota, whereas healthy infants had a typical infant *Bifidobacterium* microbiota. However, this difference was not confirmed in other studies using *Bifidobacterium*-specific polymerase chain reaction combined with denaturing gradient gel electrophoresis to compare the faecal microbiota of healthy control infants with that of IgE-associated wheezy infants [31] or IgE-associated eczematous infants [36].

The *Atopobium* cluster count, which was higher in our CMPA infants, comprises most of the *Coriobacteriaceae* species, including the *Coriobacterium* group. Bacteria of the *Coriobacterium* group are usually detected in the faeces of formula-fed infants, and *Coriobacteriaceae* species represent a numerically important [19] but often overlooked [20, 37] group. The elevated *Atopobium* cluster count in CMPA may suggest a relationship between the *Coriobacterium* group and the CMPA. However, the contribution of these bacteria to pathophysiology still needs to be elucidated.

The higher concentrations of BCSFA in the CMPA infants could be related to the intestinal inflammatory process associated with CMPA allergy. BCSFA and ammonia are considered mediators of inflammation and lesion at the intestinal level [38]. Elevation of these fatty acids has been described in patients with inflammatory bowel disease and has been associated with a specific toxicity against intestinal mucosa [27].

In conclusion, the present findings suggest that dysbiosis – i.e. changes in gut microbiota composition – may be implicated in the pathogenesis of CMPA, although no single species or genus appears to play and essential role. Further studies are required to confirm differences in faecal microbiota composition between CMPA and healthy infants and to identify any specific changes associated with the development of the disease.

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