Gut microbial metabolomics to understand allergies in early life

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Chapter 2

Current insights into cow's milk allergy in children: microbiome, metabolome and immune response – a systematic review

Based on:

Current insights into cow's milk allergy in children: microbiome, metabolome and immune response – a systematic review

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Abstract

Background: The increasing prevalence of IgE-mediated cow's milk allergy (CMA) in childhood is a worldwide health concern. There is a growing awareness that the gut microbiome (GM) might play an important role in CMA development. Therefore, treatment with probiotics and prebiotics has gained popularity. This systematic review provides an overview on the alterations of the GM, metabolome and immune response in CMA-children and animal models, including post-treatment modifications.

Method: MEDLINE, PubMed, Scopus and Web of Science were searched for studies on the GM in CMA-diagnosed children, published before March 1, 2023.

Results and conclusions: A total of 21 articles (13 on children, 8 on animal models) were included. The studies suggest that the GM, characterized by an enrichment of the Clostridia class and reductions in the Lactobacillales order and *Bifidobacterium* genus, is associated with CMA in early life. Additionally, reduced levels of short chain fatty acids (SCFAs) and altered amino acid metabolism were reported in CMA-children. Commonly used probiotic strains belong to the *Bifidobacterium* and *Lactobacillus* genera. However, only *Bifidobacterium* levels were consistently upregulated after intervention, while alterations of other bacteria taxa remain inconclusive. These interventions appear to contribute to the restoration of SCFAs and amino acid metabolism balance. Mouse models indicate that these interventions tend to restore the T_h2/T_h1 balance, increase the T_{reg} response, and/or silence the overall pro- and anti-inflammatory cytokine response. Overall, this systematic review highlights the need for multi-omics related research in CMA-children to gain a mechanistic understanding of this disease and to develop effective treatments and preventive strategies.

Keywords

infant, cow's milk allergy, gut microbiota, metabolomics, synbiotics, mouse model, immune response

1. Introduction

One of the most common food allergies in early childhood is cow's milk allergy (CMA).[1,2] Allergic reactions can be IgE-mediated, non-IgE-mediated, or a mix of both.[3] Multiple studies have shown that among the children diagnosed with CMA those with IgE-mediated reactions to cow's milk tend to have persistent symptoms more often and acquire tolerance slower than those with non-IgE-mediated reactions.[4–7] At present, infants diagnosed with CMA are placed on an elimination diet consisting of an extensively hydrolyzed formula (EHF) or, if symptoms persist, an amino-acid formula (AAF).[8] Because of the increasing evidence linking food allergies with alterations in gut microbial composition,[9,10] modifying the gut microbiome (GM) with probiotics, prebiotics or synbiotics has emerged as a promising way to prevent and treat allergies.[11] However, there is still little mechanistic understanding on how the GM influences host immune health, leading to allergies, including CMA.[12] Recent technological innovations in the field of microbiome, proteomics and metabolomics have opened new doors for research and provided opportunities to address the gap in understanding the role of GM in CMA. The objective of this systematic review is to further the understanding of the relationship between the GM and CMA, by reviewing existing studies examining microbiome, metabolome, proteome, and immune response data on IgE-mediated CMA in children and animal models.

2. Methods

This systematic review is registered in PROSPERO (CRD42021290177).

2.1 Search strategy

A search in MEDLINE, PubMed, Scopus and Web of Science was performed using the queries in **Table S1**. The search was limited to research articles published in English before March 1, 2023.

2.2 Inclusion and exclusion criteria

Human case, case-control, and intervention studies were included only if they examined children with IgE-mediated CMA aged 0-12 years. The allergy had to be medically diagnosed by either a skin prick test (SPT) or an IgE-specific test combined with a cow's milk food challenge. In studies with fecal transplantation (FT), the IgE-mediated CMA status of the donor must be confirmed by the diagnosis criteria used for human studies. For studies reporting data on groups of subjects diagnosed with different types of CMA, only the group with IgE-mediated CMA was reviewed. For animal studies, only case-control and intervention studies on models that included both sensitization and challenge steps were included. The studies were included only if they contained analytical data that examined the GM or metabolome and were excluded when they failed to meet the inclusion criteria, had unclear diagnosis, or involved antibiotic treatment.

2.3 Study selection

Titles, abstracts, and methods were screened independently by two of the authors MVS, PZ, DMH, and by a third author in case of disagreement. Subsequently, the full text of the studies marked as potentially eligible was retrieved and independently checked for eligibility by at least two of the authors MVS, PZ, DMH, and by a third author in case of disagreements or doubts.

2.4 Data extraction

For human studies, the extracted data included general study details (author, year), participant information (age, sample size), CMA diagnosis, analytical data types, data acquisition techniques, measured analytical parameters and significant results. For intervention studies, the intervention details were also extracted. If available, the age range for each group in the study was reported. When only the mean and standard deviation (sd) were available, the age was reported as mean ± sd. The results were split in two: increased and decreased variables between the compared groups. For animal intervention studies, the extracted data included general study details, model information, challenge information, intervention details, data acquisition techniques, measured analytical parameters and significant results.

3. Results

3.1 Search strategy

Our search yielded 733, 479, 512, 897 articles in respectively Scopus, PubMed, MEDLINE and Web of Science. Forty-nine studies were eligible for inclusion. **Figure 1** shows the PRISMA[13] flow diagram. Of the 49 papers, 28 were excluded after careful consideration by two authors or three in case of a disagreement or a doubt (**Table S2**).

3.2 Study findings

3.2.1 Human studies

CMA diagnosis criteria and measured parameters in human studies are summarized in **Table S3**.

3.2.1.1 Case and case-control studies

Human studies include one case and nine case-control studies (**Table 1**), among which four examined both the microbiome and metabolome,[14–17] five the microbiome,[18–22] and one the metabolome.[23] For all case-control studies, healthy controls (HC) were used except for one study[23] that considered atopic eczema/dermatitis syndrome infants as controls.

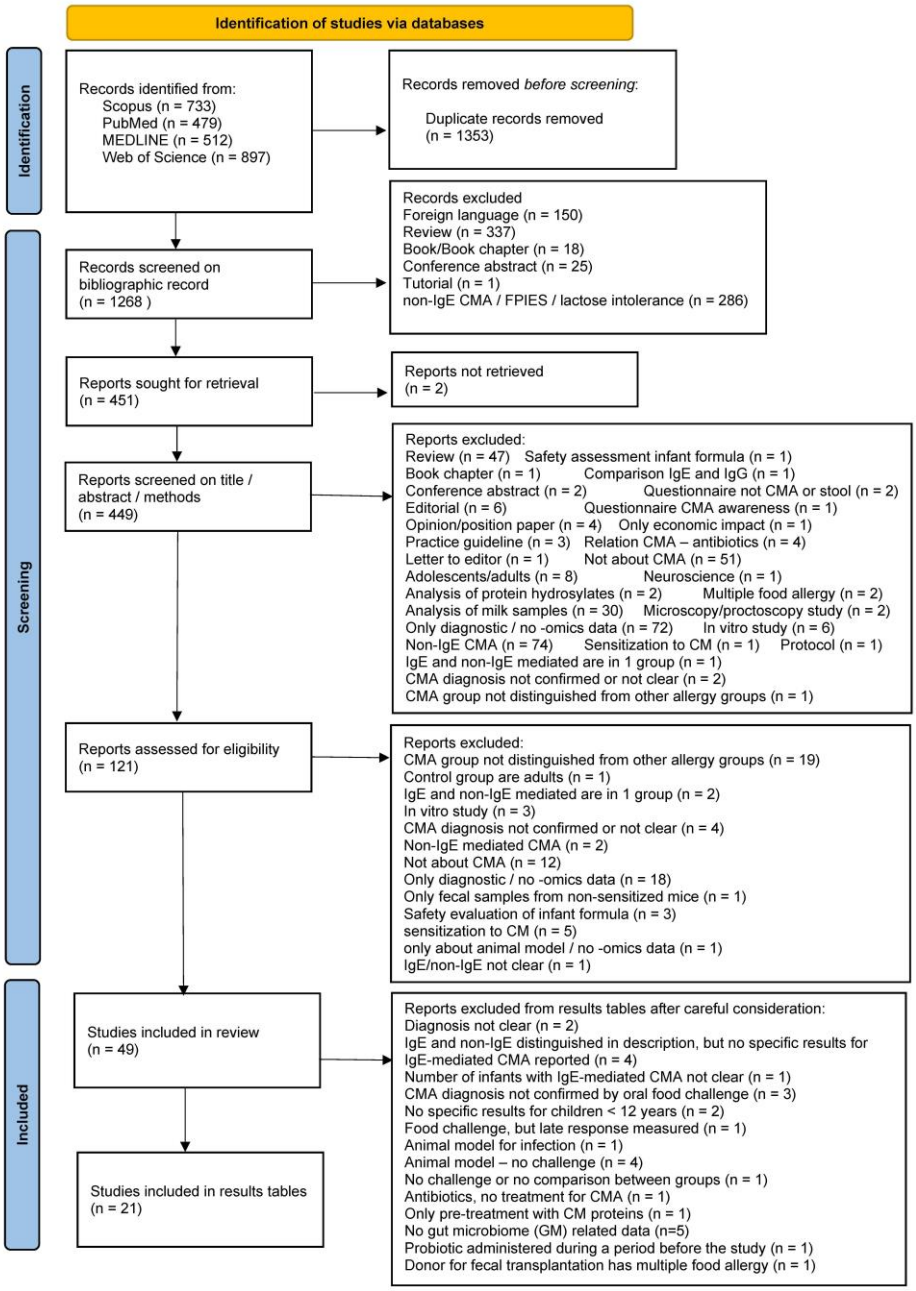

Figure 1. PRISMA flow chart for this systematic review.

GM modifications

The GM-related studies include four case-control reports,[15,19,17,20] four case-control findings in intervention studies,[14,16,18,21] and one case study.[22] Techniques applied for GM profile identification included bacteria culture[18] and 16S rRNA gene-based approaches (DGGE,[19] FISH[14,15] and gene sequencing[16,17,21,20,22]). Two studies applied specific probes to target certain bacteria groups,[14,15] and six used universal probes or primers to target the V3 region,[19] V4 region[16,22] or both.[17,20,21]

Six studies compared α- and β-diversity between CMA-group and HC, three of them noted increased[16,19] or decreased[20] Shannon α-diversity difference in the CMA-groups, and one reported β-diversity (unweighted UniFrac) difference between CMA-group and HC.[21] A single study reported a higher total bacteria count in the CMA-group.[18]

Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia were the primary reported GM phyla. Elevated abundances of the Firmicutes phylum were consistently observed in the CMA-groups.[14–19,21] These included: total Firmicutes;[17,21] the class Clostridia;[17] the families *Lachnospiraceae*[16] and *Ruminococcaceae*[16,17]; the genera *Clostridium*,[14,19] *Faecalibacterium*,[16] *Lactobacillus*,[18] *Ruminococcus*[16] and *Subdoligranulum*[19] and the species *Clostridium coccoides*[15] and *Clostridium celerecrescens*.[19] Conversely, certain Firmicutes phylum, including the genus *Granulicatella*[21] *and the families Streptococcaceae,*[16] *Enterococcaceae,*[16] and *Acidaminococcaceae,*[20] decreased in the CMA-groups. Additionally, enriched bacteria of the Firmicutes phylum, including the class Clostridia, were also observed in the infants who outgrew CMA.[22]

Bacteroidetes phylum members also showed varying changes in the CMA-groups.[14,17,19–21] These included increased levels of the *Flavobacteriaceae* family,[17] the *Bacteroides*,[14,19] and *Prevotella*[21] genera, along with reduced abundance of the *Prevotellaceae* family[20] and the *Parabacteroides* genus.[21] Furthermore, several bacteria from the Proteobacteria phylum, including the *Haemophilus*, *Actinobacillus* and *Klebsiella* genera,[21] and the *Escherichia coli* species,[19] increased in the CMA-groups. In contrast, total Proteobacteria,[17] the *Enterobacteriaceae* family,[16,18] and the *Escherichia* genus[16] decreased. In the Actinobacteria phylum, one study reported increased *Atopobium* cluster (genus) levels,[15] while *Bifidobacteriaceae* family members, including *Bifidobacterium* spp., consistently exhibited decreased abundance in the CMA-groups.[14,16,18,19] Additionally, the Verrucomicrobia phylum dropped in the CMA-group.[21]

Two studies reported certain bacteria only in the CMA group or the HC. The Clostridium *celerecrescens* species,[19] and the *Burkholderiaceae*, *Nannocystaceae*, *Shewanellaceae*, *Thermomonosporaceae* and *Flavobacteriaceae* families were reported only in the CMA group.[17] In contrast, the *Bifidobacterium bifidum* species[19] and the *Methylophilaceae* and *Dietziaceae* families were exclusively detected in the HC.[17]

Table1. Human case and case-control studies in infants/children. Abbreviations: see Table S1

Age years (y); months (m)	**Analytical techniques**	**Type of analytical data**	**Sample size** (CMA/control)	**Results: modifications in case versus control (case-control study), modifications in allergic versus tolerant (case study)**		**Reference**
				Increase	**Decrease**	
1-12 m	Bacterial culture (CFU)	Microbiome	46/46	**Baseline:** Total bacteria count, Anaerobic bacteria **After 6 months:** Anaerobes count, Lactobacilli count and proportion	**Baseline:** Yeast count **After 6 months:** Bifidobacteria count and proportion, Enterobacteria proportion, Yeast proportion	Thompson-Chagoyan *et al.*[18]
0.55 ± 0.20 y	GC-MS	Metabolomics	16/16	beta-hydroxybutyrate, adipate, isocitrate, homovanillate, suberate, tartarate, 3-indoleacetate, 5-hydroxyindoleacetate	Not reported	†Salmi *et al.*[23]
2-12 m	FISH-FC (16S rRNA gene specific probes); GC-FID	Microbiome, Metabolomics	46/46	*Clostridium coccoides* group, *Atopobium* cluster, butyrate, BCSFA	Not reported	Thompson-Chagoyan *et al.*[15]
6.5-10.4 m	FISH (16S rRNA gene specific probes); GC-MS; NMR	Microbiome, Metabolomics	18/18	*Bacteroides, Clostridium*, Total esters, ketones, alcohols, aldehydes; uridine, histidine, tyrosine, TMAO, arginine/histidine	Bifidobacteria, Total SCFAs (major difference: acetate and butyrate), pyruvate, lactic acid, threonine, proline	Francavilla *et al.*[14]
5-8 y	PCR-DGGE (V3 regions + 16S rRNA gene- specific primers)	Microbiome	12/12	GM α-diversity (Shannon diversity), *C.coccoides* diversity (Shannon diversity), *Bacteroides, Clostridium, Escherichia coli'* only detected in CMA group: *C. celerecrescens*	*Bifidobacterium* (*B.*) diversity (Shannon diversity), *B. adolescent*, *B. longum*, *B. catenulatum*, *and B. breve* Only detected in control group: *B. bifidum*	Guo *et al.*[19]

Table1. Continued

Age years (y); months (m)	**Analytical techniques**	**Type of analytical data**	**Sample size** (CMA/control)	**Results: modifications in case versus control (case-control study), modifications in allergic versus tolerant (case study)**		**Reference**
				Increase	**Decrease**	
1-12 m	qPCR-16S rRNA (V4 region), GC-FID	Microbiome	19/20	GM α-diversity (Shannon diversity), Gut microbiota evenness (Pielou's evenness), *Ruminococcaceae, Lachnospiraceae*, *Ruminococcus, Faecalibacterium*	*Bifidobacteriaceae, Streptococcaceae, Enterobacteriaceae, Enterococcaceae, Bifidobacterium, Escherichia*	Canani *et al.*[16]
5-8 y	PCR-16s rRNA (V3-V4 regions), HPLC-UV	Microbiome, Metabolomics	6/8	Firmicutes, Clostridia, *Ruminococcaceae, Subdoligranulum* only detected in CMA group: *Burkholderiaceae, Nannocystaceae*, *Shewanellaceae*, *Thermomonosporaceae, Flavobacteriaceae*	Proteobacteria only detected in control group: *Methylophilaceae, Dietziaceae,* Total SCFAs	Dong *et al.*[17]
10-15 m	PCR-16S-rRNA (V3-V4 regions), qRT-PCR	Microbiome	14/14	Firmicutes, *Haemophilus, Actinobacillus, Prevotella, Klebsiella*	Verrucomicrobia, *Parabacteroides, Granulicatella*	Mennini *et al.*[21]
4-6 m	16S-rRNA (V3-V4 regions)	Microbiome	16/34	Not reported	GM α-diversity (Shannon diversity), *Acidaminococcaceae, Prevotellaceae*	Mera-Berriatua *et al.*[20]
3-16 m	16S-rRNA (V4 region)	Microbiome	226/- (3-6m: 29/-)	Fecal microbiome at 3-6 month: *Bacteroidetes*, *Enterobacter* Metagenome functional enrichment of fatty acid metabolism.	Fecal microbiome at 3-6 month: Clostridia, Firmicutes.	Bunyavanich *et al.*[22]

†AEDS as basic disease for subjects in both case and control group, and the age is calculated by the pooled mean and sd from the age groups provided in the article

Metabolome modifications

Decreased total short chain fatty acid (SCFAs),[14,17] along with increased butyrate and total branched-chain short fatty acids (BCSFAs),[15] were reported in CMA-groups. Besides, lower pyruvate, lactate, threonine and proline, along with higher total esters, ketones, alcohol aldehydes, uridine, histidine, tyrosine, trimethylamine-N-oxide (TMAO) and arginine/histidine,[14] and elevated organic acids were reported in CMA-groups.[23]

Metabolome-microbiome associations

Two studies examined the association between the GM and the metabolome.[15,17] Positive correlations were found between the *Clostridium* genus and butyrate, the *Clostridium coccoides* species and BCSFAs, and the *Bacteroides* genus and propionate.[15] Isocaproate and BCSFAs were negatively related with the *Bifidobacterium* genus.[15] Additionally, lactate was found to be negatively correlated with *Bacteroides* genus[17] *and Clostridium coccoides* species,[15] but positively correlated with *Bifidobacterium* genus.[15]

3.2.1.2 Intervention studies

Eight intervention studies for CMA treatment were included (**Table 2**).[14,16,18,21,23–26] Two examined the GM and metabolome,[14,16] one the GM and immune response,[26] four the GM,[18,21,24,25] and one the metabolome.[23] The interventions varied across studies, including synbiotics,[25] prebiotics,[24] probiotics (species of the genus *Bifidobacterium*,[21,26] *Lactobacillus rhamnostbus* GG (LGG) species[16,23]) and different formula types.[14,18]

GM modifications

The GM profile was identified with bacteria culture,[18] FISH,[25] 16S rRNA gene sequencing with specific primers/probes[14,24,26] or targeting the V4[16] or V3-V4 regions.[21]

Alterations of the phylum Firmicutes in CMA-patients were described in five intervention studies, involving treatment with EHF,[18] lactose-supplemented EHF,[14] LGG,[16] species and strains from the *Bifidobacterium* genus.[21,26] These interventions raised Firmicutes phylum members, including the Turicibacterales order,[48] the *Lactobacillaceae* and *Lachnospiraceae* families[48] and the genera like *Lactobacillus*,[18,48] *Blautia*,[16,21] *Roseburia*,[16] *Coprococcus*,[16] *Anaerofustis*,[16] *Ruminococcus*,[21,26] *Turicibacter,*[26] and *Oscillospira*.[26] Conversely, some Firmicutes phylum members, including the Clostridia class,[14] *Christensenellaceae* family,[48] and genera like *Enterococcus*, *Streptococcus*,[21] *Anaerovibrio*, *Oscillibacter*, *Bilophila*, *Dorea* and *Roseburia*[26] decreased under treatments.

The interventions also affected the Proteobacteria phylum[21] and its members. The Betaproteobacteria class, the Burkholderiales order, the *Alcalligenaceae* family and the *Sutterella* genus increased in the treated group,[26] while some studies reported decreased levels of the Deltaproteobacteria class,[26] the *Enterobacteriaceae* family,[18] and the *Sutterella* genus.[21] In the Bacteroidetes phylum, studies reported the interventions increased levels of

the *Porphyromondaceae* family[26] and the *Prevotella* genus,[21,26] and reduced levels of the *Bacteroides* and *Prevotella* genera.[14] Additionally, the Actinobacteria phylum also underwent changes with interventions.[14,18,21,25,26]

The use of probiotic *Bifidobacterium* strains consistently elevated the *Bifidobacterium* genus.[21,25,26] Increased *Bifidobacterium* were also noticed after lactose-supplemented EHF diet.[14] In contrast, the Actinobacteria phylum[21] and its members, the genera *Bifidobacterium*,[18] *Atopobium,*[21] and *Actinomyces,*[21,26] were decreased by the treatments. The Verrucomicrobia phylum and its *Akkermansia* genus were found increased in the treatment group.[21]

In addition to the taxonomy changes, enhanced α-diversity (chao1, observed species),[26] reduced total bacteria,[24] and a decreased ratio of the *Eubacterium rectale*/*Clostridium coccoides* species[25] were reported after probiotics, pectin-based thickened AAF and synbiotics treatments, respectively.

Metabolome modifications

After the LGG-supplemented hydrolyzed whey formula (HWF) diet, CMA-patients showed increased kynurenate and decreased 3-indoleacetate.[23] Additionally, butyrate increased in LGG-supplemented extensively hydrolyzed casein (EHC) formula treated CMA-patients.[16] Meanwhile, lactose-supplemented EHF raised SCFAs, lactate, threonine, uridine, histidine, tyrosine, methionine, TMAO, phenylalanine, arginine/histidine and gamma–amino–butyrate/lysine, and lowered the total esters, ketones, alcohols, aldehydes and valine/isoleucine in CMA-patients.[14]

Immune response

The single intervention study reporting findings on the immune response showed that *Bifidobacterium bifidum* reduced allergy symptoms, lowered serum IgE and raised IgG_2 levels in CMA-patients.[26] The IgG_2 and IgE were respectively positively and negatively correlated with GM α-diversity (Chao1 index, observed species, community diversity index, Shannon index). The intervention decreased the pro-inflammatory cytokines TNFα, IL-1β and IL-6 and increased the anti-inflammatory cytokine IL-10 as well.[26]

CMA outcome

Four out of eight intervention studies discussed CMA tolerance or allergic symptoms improvement between treatment and control.[16,24–26] Two studies noted significant improvement in allergic symptoms after treatment,[24,26] and one reported five out of 12 infants in the treated group outgrew CMA after six months, compared to none in the control group.[16]

Table 2. Characteristics of studies that compare CMA infants/children before and after intervention (intervention study). Abbreviations: see Table S1

Age years (y); months (m)	**Analytical techniques**	Type of analytical data	**Sample size** (treatment/ control)	**Intervention detail**				**Results: modifications in treatment versus control**		**Reference**
				Duration (months)	**Comparison groups**	**Control diet** (Basic formula (BF))	**Treatment diet** (BF + intervention)	**Increase**	**Decrease**	
0.55 ± 0.20Y	GC-MS	Metabolomics	9/5	1	Treatment vs control	HWF	HWF with LGG	Kynurenate	3-indoleacetate	Salmi *et al.*[23]
2-12 m	Bacteria culture (CFU)	Microbiome	46/46	6	CMA subjects before intervention	-	EHF	*Lactobacilli*	Enterobacteria Bifidobacteria	Thompson-Chagoyan *et al.*[18]
6.5-10.4 m	FISH (16S rRNA-specific probes), GC-MS, NMR	Microbiome, Metabolomics	16/16	2	CMA subjects before intervention	-	EHF with 3.8% lactose	*Bifidobacteria,* LAB, SCFAs, lactate, threonine, uridine, histidine, tyrosine, methionine, TMAO, phenylalanine, arginine/histidine, c–amino butyrate/lysine	*Atopobium*, *Bacteroides/Prevote*, Clostridia and sulfate-reducing bacteria, total esters, ketones, alcohols, aldehydes, valine/isoleucine	Francavilla *et al.*[14]
6.2 ± 4.3m	qPCR (16S rRNA-specific primers and probes)	Microbiome	23/17	3	Treatment vs control	RAAF	TAAF	Not reported	Total bacteria count	Dupont *et al.*[24]
1-12 m	qPCR-16S rRNA (V4 region), GC-FID	Microbiome; Metabolomics	12/7	6	Treatment vs control, CMA subjects before intervention	EHC formula	EHC formula with LGG	**er vs before intervention:** *Blautia, Roseburia, Coprococcus* **Compared to control group:** *Roseburia,Anaerofustis*. Butyrate	Not observed	Canani *et al.*[16]

Table 2. Continued

Age years (y); months (m)	**Analytical techniques**	Type of analytical data	**Sample size** (treatment/ control)	**Intervention detail**				**Results: modifications in treatment versus control**		**Reference**
				Duration (months)	**Comparison groups**	**Control diet** (Basic formula (BF))	**Treatment diet** (BF + intervention)	**Increase**	**Decrease**	
0.5-12 m	ELISA qPCR (16S rRNA-specific primers)	Microbiome, Immune response	123/121	6	Treatment vs control	-	*B. bifidum* TMC3115	After 6 months: IL-10, total IgG_2, GM α-diversity (chao1 index, observed species), Bifidobacteriales, *Bifidobacterium*, *Lactobacillaceae Lactobacillus, Turicibacter, Turicibacterales, Betaproteobacteria, Sutterella, Burkholderiales, Alcalligenaceae, Porphyromondaceae, Parabacteroides,Ruminococcus,Oscillospira, Lachnospira*	After 6 months: TNFα, IL-1, IL-6, IL-10, total IgE, *Anaerovibrio, Christensenelaceae, Oscillibacter, Bilophila, Dorea Roseburia*) Desulfovibrionales, Deltaproteobacteria, *Proteobacteria, Actinomyces)*	Jing *et al.*[26]
10-15 m	PCR-16S rRNA (V3-V4 regions), qRT-PCR	Microbiome	14/14	1	CMA subjects before intervention	-	probiotic mix: *B. breve* M-16V, *B. longum subsp. longum* BB536, *B. longum subsp. Infantis* M-63	Verrucomicrobia, Proteobacteria, *Akkermansia, Prevotella, Ruminococcus, Blautia, B. longum subspecies infantis*	Actinobacteria, *Actinomyces, Enterococcus, Streptococcus, Sutterella*	Mennini *et al.*[21]
<13 m	FISH (16S rRNA s-specific probes	Microbiome	80/89	12	Treatment VS control	AAF	Synbiotics: oligofructose, inulin, *B. breve* M-16V	After 6 and 12 month: bifidobacteria	After 6 month: ER/CC	Chatchatee *et al.*[25]

Table 3. CMA intervention studies with animal models. Abbreviations: see Table S1

Groups		Platforms	Results†		Reference
Case/Intervention	**Control**		**Microbiome/Metabolome**	**CMA outcome & Immune response**	
G1: *L. rhamnosus* **G2:** *B. longum subsp. Infantis* **G3:** *L. salivarius* **G4:** *B. bifidum* **G5:** *L. gasseri* **G6:** *B. animalis subsp. lactis*	**AC**: PBS	**Immunoglobulins** ELISA **Cytokines** IA (ex-BLG) **mRNA expression** q-PCR **Microbiome** qPCR (16s rRNA-specific primers); bacteria culture	**Microbiome** Total bacteria ↓ G1, G2, G3, G4, G5 *Clostridium* cluster IVa ↑ G1, G6 *Staphylococci* abundance ↑ G1 *C. leptum*↑ G1, G6 *Prevotella*↑ G6 *C. leptum* ↓ G2, G3, G4, G5 *Prevotella*↓ G2, G3, G4, Lactobacillus ↓ G2, G3, G4, G5 *Clostridium* cluster I/II↓ G2, G3, G5 Clostridium cluster XI ↓ G2, G3, G4 *C. coccoides*↓ G2, G3, G4, G5 *Enterococcus* ↓ G2, G3, G4, G5 *Enterococcus* ↓ G1	**Allergy markers** mMCP-1 ↓ G1, G2, G3 **Immunoglobulins** BLG-sIgE ↓ G1, G2, G3 BLG-sIgG_1/sIgG_{2a}↑ G1, G2, G3, G4, G6 **Cytokines** IL-4 ↓ G1, G2, G3, G4 (spleen, MLN) IFN-γ ↑ G1, G2, G6 (spleen) IFN-γ ↓ G3, G4 (spleen) IFN-γ ↑ G6 (MLN) IL-10 ↑ G1, G2, G6 (spleen) IL-10 ↑ G1, G5, G6 (MLN) **mRNA expression** IL-4 ↓ G2 IL-10, GATA3, RORγT ↓ G2, G3 FOXP3 ↑ G2, G3 IL-17a ↑ G1, G2, G3	Neau *et al.*[31]
G1 pWH **G2/G3:** pWH + short(G2)/long (G3) scGOS/lcFOS (9:1) **G4/G5:** pWH + short (G4)/long (G5) scGOS/lcFOS (9:1) + pAOS	**TC:** W **AC:** PBS	**Microbiota** PCR (16S rRNA V3-V4 regions) **Immunoglobulins** ELISA	**Microbiome** *Prevotella* ↑ G3, G4, G5 vs G1 *Lactobacillus* ↓ G5 vs G1	**Allergy markers** mMCP-1 ↓ G1, G5 vs AC TSLP ↓ G1 vs AC AASR ↓ TC, G1, G2, G4, G5 vs AC SAS & body-T ↓ TC, G2 vs AC	Kleinjans *et al.*[32]

Table 3. Continued

Groups		Platforms	Results†		Reference
Case/Intervention	Control		Microbiome/Metabolome	CMA outcome & Immune response	
G1: *L. rhamnosus* **G2:** *B. longum subsp. Infantis* **G3:** *L. salivarius*	**AC**: PBS	**Microbiome** PCR -16S rRNA (V3-V4 regions) **Metabolome** GC-FID, UPLC-MS/MS **Immunoglobulins** ELISA **Cytokines** IA (ex-BLG) **mRNA expression** qPCR	**Metabolome** Kynurenine, N-acetylkunurenine ↓ G1, G2, G3 **Microbiome** Richness (OTU number) ↑ G1 Beta diversity ↑ G1, G2, G3 *Prevotellaceae* ↑ G1, G2, G3 *Marinifilaceae* ↑ G1, G2 *Ruminococcaceae* ↑ G1 *Helicobacteraceae* ↓ G1 *Ruminococcaceae* ↓ G2 *Lachnospiraceae* ↓ G1, G2, G3 *Deferribacteraceae* ↓ G1, G2 *Clostridiaceae* ↓ G1 *Peptococcaceae* ↓ G1, G3 *Burkholderiaceae* ↓ G1 *Anaeroplasmataceae*↓ G2	**Cytokines** GM-CSF, IL-2, IFN-γ, IL-4 ↓ G1, G2, G3 IL12p70 and IL10 ↓ G1 IL-5 ↓ G2, G3 IL17A ↓ G1, G3 **mRNA expression** FOXP3, IL-10 ↑ for G1 and G3 TGFβ ↑ G1, G2, G3	Esber *et al.*[28]
G1: mix of W peptides (PepMix) **G2:** scFOS and lcFOS (9:1) + *B. breve* M-16V (FF/Bb) **G3:** PepMix + FF/Bb	**TC:** W **AC:** PBS	**Immunoglobulins** ELISA **Metabolites** GC-FID **Lymphocytes** FC **Cytokines** IA (ex-W)	**Metabolites** acetate, butyrate ↑ G2 butyrate ↑ G2 vs G3, TC vs AC	**Allergy markers** AASR ↓ G3, TC vs AC SAS ↓ TC vs AC **Lymphocytes** (SI-LP) T_h1/T_h2 ↑ G3, TC T_{reg}, T_h17 ↑ AC vs TC **Cytokines** (spleen) IFN-γ, IL-17A, IL-13, IL-5, IL-10 ↓ G3 vs G1 & TC vs AC IL-10 ↑ G3	Kostadinova *et al.*[33]

Table 3. Continued.

Groups		Platforms	Results†		Reference
Case/Intervention	**Control**		**Microbiome/Metabolome**	**CMA outcome & Immune response**	
G1: mix of W peptides (**PepMix)** **G2:** scFOS and lcFOS (9:1) + *B. breve* M-16V (**FF/Bb)** **G3:** PepMix + FF/Bb	**TC:** W **AC:** PBS	**Metabolites** GC-FID **Lymphocytes** FC **mRNA expression** qPCR **Immunohistochemistry**	<u>Part 1: Post-oral tolerance</u> **Metabolites** butyrate ↑ G3 cs G1 propionate ↑TC, G2, G3 vs AC Positive correlation: propionate and FOXP3+ (colon)	**Allergy markers** AASR ↓ G3, TC vs AC AASR ↑ G1, G2 vs G3 SAS ↓ TC vs AC <u>Part 1: Post-oral tolerance</u> **Lymphocytes** FOXP3+/GATA3+, T_{regs}/T_{effs} ↑ G3 vs AC, G3 vs G2, G3 vs G1 (MLN) T_{regs} ↓ G3 vs AC, G3 vs G2, TC vs AC (spleen) CD25+ ↓ G3 vs G2 **DC** (SI-LP) $CD8\alpha^{-}CD11b^{+}/CD8\alpha^{+}CD11b^{-}$, $CD11b^{+}CD103^{-}$ ↑ G3 $CD8\alpha^{+}CD11b^{-}$ ↓ G1 **mRNA expression** FOXP3/GATA3 ↑ G3 (PP) FOXP3/RORγT ↑ G3 vs AC, G3 vs G2, G3 vs G1 (PP) TGF-β ↑ G3 vs G2 (proximal SI) TGF-β ↓ G1 (colon) IL-22 ↑ G3 vs AC, G3 vs G1 (PP) IL-22 ↑ for G3 vs G1 (middle SI) IL-22 ↑ G2 vs AC & G2 vs G3 (colon) Galectin 9 ↓ TC Tbet/GATA3 ↓ G1 vs AC, G1 vs G3 (colon) <u>Part 2: Post-challenge</u> **Lymphocytes** (SI-LP) CD25+ Tcells ↑ G3 CD25+ Tcells ↑ G3 vs G2 T_{reg} ↑ G1 **mRNA expression** (PP) Tbet/GATA3 ↑ G3 IFN-γ/IL-13 ↑ G3 vs AC & G3 vs G2	Kostadinova *et al.*[34]

Table 3. Continued

Groups		Platforms	Results†		Reference
Case/Intervention	**Control**		**Microbiome/Metabolome**	**CMA outcome & Immune response**	
G1: M-C57BL/6J **G2**: M BALB/cJ **G3**: F-C57BL/6J **G4**: F-BALB/cJ	**S:** sham control (sex and strain matched to G1, G2, G3,G4 separately)	**Immunoglobulins** ELISA **Cytokines, chemokines, and acute phase proteins:** IA **Microbiota** 16S rRNA sequencing (8 regions)	**Microbiome** α-diversity ↑ G4 (Simpson and Shannon indices) α-diversity↓ G1(Simpson index) Bacteroidetes↑G3 Patescibacteria↑G3 Verrucomicrobia↓ G1 Proteobacteria↓ G1 Actinobacteria↓G3	**Allergy markers** Body-T ↓ G2 vs S, G4 vs S, G4 vs G3 SAS ↑ G2 vs S, G4 vs S, G4 vs G3 **Immunoglobulins** sIgE ↑ G2 vs S, G1 vs S, G4 vs S, G4 vs G3 $sIgG_1$ ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 $sIgG_{2a}$ ↑ G2 vs S, G2 vs G1, G4 vs S, G4 vs G3 **Cytokines, chemokines, and acute phase proteins:** G1 vs S: ↑ in CCL1, CSF1, IL-13, CCL17, IL-21, FGF2, CCL12, IL-10, CCL9 G2 vs S: ↓ IL-1β, IL-13, CSF2, TNFRSF1A G4 vs S: ↑ IL-15, TNFRSF1B, ICAM-1	Smith *et al.*[30]
G1: CMA	**S**: Sham control	**Microbiome** PCR-16S rRNA (V3-V4 regions) **Immunoglobulins** ELISA **Cytokines** ELISA **mRNA expression** qPCR **Metabolome** GC-FID, RP, HILIC-MS/MS	**Microbiome** *Barnesiella*↑ *Clostridium*_XIVa↑ *Lactobacillus*↓ *Parvibacter*↓ Only observed in sham mice: *Bosea*	**Allergy markers** Body-T ↓ G1 vs S SAS ↑ G1 vs S Histamine ↑ G1 vs S mMCP-1 ↑ G1 vs S **Immunoglobulins** whey-sIgE, $sIgG_1$, $sIgG_{2a}$ ↑ G1 vs S **Cytokines** IL-6, IL-10 ↑ G1 vs S **mRNA expression** IL-8, IL-33, mTOR mRNA ↑ G1 vs S	Cao *et al.*[27]

Table 3. Continued

Groups		Platforms	Results†		Reference
Case/Intervention	**Control**		**Microbiome/Metabolome**	**CMA outcome & Immune response**	
G1: CMA-FT **G2:** *Anaerostipes caccae*-FT	**B-HC**: breast-fed HC-FT **F-HC:** formula-fed HC-FT	**Microbiome** PCR -16S rRNA (V4 region) **Immunoglobulins** ELISA **Transcriptome** RNA-seq, qPCR	**After fecal colonization before sensitization:** **Microbiome** G1 vs F-HC: *Enterococcus*↑ *Barnesiellaceae*↑ *Ruminococcus*↑*Ruminococcaceae*↑ *Coprobacillus* ↑ *Clostridiaceae* ↑ *Clostridiales* ↑ *Blautia* ↑ *Parabacteroides*↑ *Lachnospiraceae*↓ *Erysipelotrichaceae*↓ *Enterobacteriaceae*↓ *Streptococcus*↓*Enterobacteriaceae*↓ *Salmonella*↓*Anaerostipes caccae* ↓ **Transcriptome** **G1 vs F-HC:** (Mroh7, Cntn1, Slc9b2, Letm2, Acot12, Abcc2, Cyp3a59, Cyp2b10, Lrrn1, Me1, Akr1c19, Gstm1, Ces1f) ↑ (Tgfbr3, Acta1, Ror2, Slc22a13, Fbp1, Apcdd1) ↓	**Allergy markers** mMCP-1 ↑ G1, G4 vs HC mMCP-1 ↓ G2 vs G1 **Immunoglobulins** BLG-specific IgE, IgG1↑ G1 vs HC **Cytokines** IL-13, IL-4 ↑ G1 vs G2 **Transcriptome** Tgfbr3 ↓ G1 vs G2, G1 vs HC Ror2 ↓ G1, G2 vs HC Ror2, Tgfbr3 positively correlated to *Lachnospiraceae*	Feehley *et al.*[29]

3.2.2 Animal studies

The animal studies include two studies on the GM, metabolome and immune response,[27,28] four on the GM and immune response[29–32] and two on the metabolome and immune response[33,34] (**Table 3**). All animal models were on mice, details are provided in **Tables S4-S6**.

GM modifications

Three interventions,[28,31,32] two case-controls[27,30] and one FT[29] study reported GM modifications. Bacteria were identified using 16S rRNA gene-targeted primers, which targeted group/species-specific bacteria[31] or certain hypervariable regions (V3-V4,[27,28,32] V4[29] and eight other regions[30]).

In two studies comparing GM changes between CMA- and sham mice,[27,30] one observed increased Simpson α-diversity in CMA-male-C57BL/6J mice but decreased Simpson and Shannon α-diversity in CMA-female-BALB/cJ mice.[30] Regardless of the strain and gender, the β-diversity (Bray-Curtis) was significant different between the two groups.[30] Apart from the gender and strain-specific α-diversity difference, CMA-mice showed enrichment in the phyla Bacteroidetes and Patescibacteria (female-C57BL/6J) but reduction in the phyla Verrucomicrobia, Proteobacteria (male-C57BL/6J) and Actinobacteria (female-C57BL/6J).[30] Compared to mice colonized with feces from healthy children (healthy-colonized mice), a FT study reported that mice with feces from CMA children (CMA-colonized mice) had higher abundances of the Clostridiales order and the *Clostridiaceae, Ruminococcaaceae* and *Barnesiellaceae* families*,* along with lower levels of the *Lachnospiraceae, Erysipelotrichaceae* and *Enterobacteriaceae* families*.*[29] At the genus level, the CMA-mice exhibited higher *Barnesiella* and *Clostridium*_XIVa,[27] and CMA-colonized mice had enhanced *Enterococcus, Ruminococcus, Coprobacillus, Blautia* and *Parabacteroides.*[29] In contrast, the *Lactobacillus*, *Parvibacter*,[27] *Streptococcus,* and *Salmonella*[29] genera, as well as *Anaerostipes caccae* species[29] decreased in CMA and CMA-colonized mice. Additionally, the *Bosea* genus was absent in CMA-mice.[27]

Species and strains of the *Lactobacillus* and *Bifidobacterium* genera were used as probiotic in CMA-mouse models.[28,31] One study reported that five out of six probiotic strains reduced the total bacteria.[31] Another found significant differences in GM β-diversity (Bray-Curtis, UniFrac) between control and treated groups but only the *Lactobacillus rhamnosus* species increased GM richness.[28] At the family level, it was reported that *Prevotellaceae* and *Marinifilaceae* increased, whereas *Helicobacteraceae*, *Lachnospiraceae*, *Deferribacteraceae*, *Clostridiaceae*, *Peptococcaceae* and *Burkholderiaceae* decreased after taking at least one probiotic.[28] Interestingly, the *Ruminococcaceae* family increased with *Lactobacillus rhamnosus* treatment but decreased with *Bifidobacterium longum subsp. infantis* treatment.[28] Furthermore, one study found that probiotic treatments with *Lactobacillus rhamnosus* and *Bifidobacterium animalis subspecies lactis* increased the *Clostridium* cluster IVa genus and the *Clostridium leptum* species.[31] Conversely, more than

three probiotic strains decreased the *Lactobacillus*, *Clostridium* cluster I/II, *Clostridium* cluster XI, *Enterococcus* and *Prevotella* genera, as well as the *Clostridium Coccoides* and *Clostridium Leptum* species.[31] Additionally, it was reported that prebiotic administration with partially hydrolyzed whey reduced the *Lactobacillus* genus and increased the *Prevotella* genus.[32]

Metabolome modifications

Two studies examined fecal SCFAs in CMA-mice with and without synbiotic intervention.[33,34] They reported enhanced acetate,[33] butyrate[33] and propionate[34] with synbiotic diet. However, one study only observed reduced kynurenine and N-acetylkunurenine in probiotic-treated mice.[28] Additionally, a FT study compared ileal transcription signatures between CMA and healthy-colonized mice.[29] They found upregulated metabolism of monocarboxylic acid, arachidonic acid, linoleic acid and pyruvate in CMA-colonized mice, while increased carbohydrate metabolic process in healthy-colonized mice.[29]

CMA outcome and immune response

Among all animal studies only Feehley *et al.*[29] and Kostadinova *et al.*[34] correlated the immune response to the GM. Feehley *et al.*[29] reported that growth factor TGF-β receptor and ROR2 genes in CMA-colonized mice was positively correlated with *Lachnospiraceae* family.[29] Meanwhile, Kostadinova *et al.*[34] showed that propionate was positively correlated with FOXP3+ cell frequency in the colon.[34]

All intervention studies reported immune response data which relates to the treatment outcome.[28,31–34] Unlike post-sensitization,[28] pre-sensitization[31] intake of *Lactobacillus salivarius*, *Lactobacillus rhamnosus* and *Bifidobacterium longum subspecies infantis* successfully lowered the mast cells degranulation marker mucosal mast cell protease-1 (mMCP-1)[35] and BLG-specific IgE.[31] All strains lowered the IL-4 secretion and the BLG-specific $sIgG_1$-to-$sIgG_{2a}$ ratio[31] which indicates the overall T_h2-to-T_h1 response.[36] The rest of the responses were strain-dependent. *Lactobacillus rhamnosus* and *Bifidobacterium longum subspecies infantis* increased T_h1 IFN-γ and T_{reg} IL-10 secretion in stimulated splenocytes, whereas *Lactobacillus salivarius* declined IFN-γ secretion.[31] Post-challenge administration of those probiotic strains predominantly induced regulatory response.[28] All strains significantly increased TGF-β expression, while *Lactobacillus rhamnosus* and *Lactobacillus salivarius* interventions also increased FOXP3 and IL-10 expression. The post-sensitization intake resulted in overall cytokine suppression as well. The reduction in granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-2, and IL-4 was common among the strains, while IL12p70, IL-10, IL-5 and IL-17A was strain-dependent.[28]

Kostadinova *et al.*[33,34] reported that synbiotic intake alone did not alleviate the acute allergic skin response but its combination with T cell-epitope-containing BLG peptides (PepMix) did.[33,34] Notably, the combined diet reestablished the lost T_h1/T_h2 balance as evidenced by the lymphocyte distribution in the small intestine lamina propria[33] as well as the increased

transcription factor (Tbet/GATA3) and cytokine (IFN-γ/IL-13) gene expression in the Peyer's Patches (PP).[34] Right after the intervention the immune response was predominantly regulatory. It was characterized by an increase in the mRNA expression of FOXP3 over the GATA3 and RORγT in the PP, as well as higher FOXP3+ over GATA3+ and T_{reg} over T_h cell frequencies in mesenteric lymph node.[34] Synbiotic addition had a site-dependent effect on IL-22 mRNA expression and also silenced the whey-stimulated splenocyte secretion of cytokines (IL-10, IL-5, IL-13, IL-17A, IFN-γ) which were induced by the PepMix intake.[33] Kleinjans *et al* showed that the effect of prebiotics on allergic symptoms varied with the composition and treatment duration.[32]

4. Discussion and conclusion

In general, no clear conclusion can be drawn about the GM diversity modification in CMA children, because of limited data on β-diversity[21,30] and discordant results regarding α-diversity in both human[16,19,20] and animal[30] studies.

Taxonomic findings showed that the *Bifidobacteriaceae* family, including *Bifidobacterium* spp., were consistently reported lower in CMA-children.[14,16,18,19] This result aligns with the consensus on the protective function of *Bifidobacterium* spp. in early life.[37,38] Another noteworthy observation concerning GM in CMA-children is the consistent increase of the Firmicutes phylum,[14–19,21] primarily associated with the Clostridia class. Conversely, decreased levels of bacteria of the Lactobacillales order were observed.[16,21] The trends of Firmicutes alterations align with the findings of an animal study which reported higher *Clostridium* cluster XIVa and lower *Lactobacillus* genus in CMA-mice.[27] However, CMA and healthy-colonized mice were both characterized with bacteria from the Clostridia class, with *Anaerostipes caccae*, a clostridial species, showing protective effects against CMA.[29] Additionally, infants who resolved CMA were reported to have enriched Clostridia class at 3-6 months.[22] Discordant results have also been reported regarding the protective or detrimental effect of the Clostridia class in food allergy.[39,40] Therefore, despite the conflicting findings of the Clostridia class in this review, we lean towards suggesting that GM with enriched Clostridia class, reduced Lactobacillales order and reduced *Bifidobacterium* genus is associated with CMA in early-life.

Various intervention approaches, including probiotics, prebiotics and synbiotics, were applied to restore the balance of GM and the metabolome in CMA-children. Elevated *Bifidobacterium* genus was consistently observed post-treatment with *Bifidobacterium* strains as probiotics[21,25,26] or after lactose-supplemented EHF treatment.[14] However, the impact on the Lactobacillales order in both CMA-children and CMA-mice was less clear. Increased levels of the *Lactobacillaceae* family were reported with *Bifidobacterium*-specific probiotics[26] and EHF in CMA-children,[18] while decreased *Enterococcus* and *Streptococcus* genera were noted in *Bifidobacterium*-treated CMA-children.[21] Additionally, decreased levels of *Lactobacillus* genus were reported in CMA-mice treated with *Bifidobacterium* and *Lactobacillus*-specific probiotics.[31,32] Similarly, the effect on the Clostridia class varied.

Higher levels of its members were reported in CMA-children and mice treated with probiotics.[16,21,26,28,31] Meanwhile, reduced Clostridia class members also noted in CMA-children treated with lactose-supplemented EHF or probiotics,[14,26] and in CMA-mice treated with probiotics.[28,31] Therefore, it is clear that the enhancement of *Bifidobacterium* after *Bifidobacterium*-specific treatment was commonly reported, however the treatment effect on other bacteria remain inconclusive. Despite the uncertainty of most GM profile modifications, there are studies which reported improved allergic symptoms or a high resolution rate in CMA-children treated with probiotics or prebiotics.[16,24,26]

In addition to GM modifications, CMA-children were reported to have decreased total SCFAs[14,16] and altered amino acids and nucleotides levels.[14,23] These findings are consistent with a recent review on the metabolic changes in children with IgE-mediated food allergies,[41] and these metabolome changes appear to be restored with interventions. Increased SCFAs and balanced amino acids were reported after treatment with LGG or lactose-supplemented EHF.[14,23] Enhanced levels of acetate,[33] butyrate,[33,34] and propionate[34] were also reported in synbiotic-treated CMA-mice.

This systematic review provides an overview of the modifications of the GM, metabolome, and immune response in IgE-mediated CMA-children and CMA animal models. Comparing microbiome data between studies is challenging due to methodological variations, diverse intervention approaches, and the reporting of different taxonomic levels. Consequently, only general conclusions can be drawn based on family or higher taxonomic levels. Meanwhile, insights into metabolomics are restricted by limited scope of studied metabolites. Thus, future work should examine broader range of metabolites known to be crucial in the crosstalk between the GM and host's immune system[41,42] and use untargeted metabolomics as hypothesis-generating strategy. Only a single human study reported microbiome and immune response data and their relationship.[26] Similarly, only a single animal study correlated transcriptomics and GM data,[29] including genes related to the immune response. Therefore, there is a need for both human and animal studies on the correlation of the GM to the immune response. Future animal studies can build on the general treatment outcome findings in the review, namely overall cytokine silencing,[28,33] restoration of the T_h2/T_h1 balance,[31,33,34] and induction of regulatory response.[28,31,34] Moreover, future work can focus on parameters already connected to allergic tolerance acquisition in human, such as induction of T_{reg} response, the production of TGF-β, IgG_4, IgA.[43] No proteomics studies met our inclusion criteria, but a study on the fecal microbiome and metaproteome relationships in CMA-children has been published after our inclusion date.[44] Overall, discussions on multi-omics connections are rare in the reviewed studies, and none of the studies reported shotgun meta-genomics, meta-transcriptomics, or meta-proteomics for microbiome function information. Therefore, there is a clear need for more comprehensive multi-omics studies to gain a better mechanistic understanding of CMA in early life. These efforts would eventually lead to the development of better and effective treatment and preventive strategies.

Author contributions

MVS: Formal Analysis, Investigation, Writing – Original Draft Preparation; **PZ**: Formal Analysis, Investigation, Writing – Original Draft Preparation; **ACH**: Supervision, Writing – Review & Editing; **RGvdM**: Investigation, Writing – Review & Editing; **CB**: Conceptualization, Funding Acquisition, Investigation, Supervision, Writing – Review & Editing; **DMH**: Formal Analysis, Investigation, Supervision, Writing – Review & Editing

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The authors declare that they have no known conflicts of interest.

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Supplementary Materials

Table S1. Abbreviations

Abbreviation	Full name/defination
16S rRNA	16S ribosomal ribonucleic acid
AAF	amino acid formula
AASR	acute allergic skin response (ear swelling)
AC	allergic control
AEDS	atopic eczema/dermatitis syndrome
BCSFAs	branched-chain short fatty acids
BLG	beta-lactoglobulin
body-T	body temperature
CFU	colony-forming unit
CM	cow's milk
CMA	cow's milk allergy
DC	dendritic cells
DGGE	denaturing gradient gel electrophoresis
DBPCFC	Double-blind, placebo-controlled food challenge
EHF	extensively hydrolyzed formula
ELISA	Enzyme-linked immunosorbent assay
ER/CC	Eubacterium rectale/Clostridium coccoides
ex-BLG	ex-vivo res-stimulation with BLG
ex-W	ex-vivo res-stimulation with whey
F	female
FC	flow cytometry
FF/Bb	short and long chain FOS and B. breve M-16V
FISH	fluorescent in situ hybridization
FOS	fructo-oligosaccharides
FOXP3	forkhead box P3
FT	fecal transplantation
G	group
GATA3	GATA Binding Protein 3
GC-FID	GC-flame ionization detector
GC-MS	gas-chromatography-mass spectrometry
GM	gut microbiome
GM-CSF	Granulocyte macrophage colony-stimulating factor
GOS	galacto-oligosaccharides
HC	healthy controls
HILIC	Hydrophilic interaction chromatography
HPLC-UV	high-performance liquid chromatography-ultraviolet detector
HWF	hydrolysed whey formula
IA	immunoassay (other than ELISA)
i.p.	intraperitoneal
i.g.	intragastric
i.d.	intradermally
IEC	Intestinal epithelial cell(s)
IFN-γ	Interferon-gamma

Abbreviation	Full name/defination
Ig(s)	immunoglobin(s)
IL	interleukin
LAB	lactic acid bacteria
lcFOS	long chain fructo-oligosaccharides
LGG	Lactobacillus rhamnosus GG
LP	lamina propria
M	male
MLN	mesenteric lymph node
mMCP-1	mucosal mast cell protease-1
MS	mass spectrometry
MS/MS	Tandem mass spectrometry
NMR	nuclear magnetic resonance
OTU	operational taxonomic unit
pAOS	pectin-derived acidic oligosaccharide
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PP	Peyer's Patches
qPCR	quantitative PCR
qRT-PCR	quantitative real-time PCR
RAAF	reference amino acid formula
Ror2	Receptor Tyrosine Kinase Like Orphan Receptor 2
RORγT	retinoid-Related Orphan Receptor gamma t
RP	reverse phase
SAS	systematic anaphylaxis scores
SCFAs	Short-chain fatty acids
scFOS	short chain FOS
scGOS	short chain galacto-oligosaccharides
sd	standard deviation
SI	small intestine
sIg	specific Immunoglobulin
SI-LP	small intestine lamina propria
sp.	single unnamed species (of a certain genus)
spp.	multiple species (of a certain genus)
SPT	skin prick test
TAAF	thickener amino acid formula
Tbet	T-box transcription factor
TC	tolerant control
Tgfbr3	Transforming growth factor beta receptor III
TGF-β	Transforming growth factor beta
T_h	T helper cell
T_{eff}	effector T cells
TMAO	trimethylamine-N-oxide
TNFα	tumor necrosis factor alpha
T_{reg}	T regulatory cell
TSLP	thymic stromal lymphopoietin

Abbreviation	Full name/defination
UPLC-MS/MS	ultra-performance liquid chromatography with tandem mass spectrometry
W	whey
Pre-S	pre-sensitization
Post-S	post-sensitization
WS	whole study
wk	week(s)

Table S2. Search queries

Database	Search query
MEDLINE	((((cow*.ti. OR cow*.ab. OR cow*.kw. OR cow*.kf.) AND (milk.ti. OR milk.ab. OR milk.kw. OR milk.kf.)) AND ((allerg*.ti. OR allerg*.ab. OR allerg*.kw. OR allerg*.kf.) OR (hypersensitiv*.ti. OR hypersensitiv*.ab. OR hypersensitiv*.kw. OR hypersensitiv*.kf.))) OR milk hypersensitivity.sh.) AND ((microb*.ti. OR microb*.ab. OR microb*.kw. OR microb*.kf.) OR (microflora.ti. OR microflora.ab. OR microflora.kw. OR microflora.kf.) OR (16S*.ti. OR 16S*.ab. OR 16S*.kw. OR 16S*.kf.) OR (bifido*.ti. OR bifido*.ab. OR bifido*.kw. OR bifido*.kf.) OR (bacter*.ti. OR bacter*.ab. OR bacter*.kw. OR bacter*.kf.) OR (lachno*.ti. OR lachno*.ab. OR lachno*.kw. OR lachno*.kf.) OR (rumino*.ti. OR rumino*.ab. OR rumino*.kw. OR rumino*.kf.) OR (veillo*.ti. OR veillo*.ab. OR veillo*.kw. OR veillo*.kf.) OR (entero*.ti. OR entero*.ab. OR entero*.kw. OR entero*.kf.) OR microbiota.sh. OR bifidobacterium.sh. OR bacteroidaceae.sh. OR bacteroides.sh. OR ruminococcus.sh. OR veillonellaceae.sh. OR veillonella.sh. OR enterobacteriaceae.sh.) AND ((child*.ti. OR child*.ab. OR child*.kw. OR child*.kf.) OR (infant*.ti. OR infant*.ab. OR infant*.kw. OR infant*.kf.) OR (baby.ti. OR baby.ab. OR baby.kw. OR baby.kf.) OR (babies.ti. OR babies.ab. OR babies.kw. OR babies.kf.) OR (toddler*.ti. OR toddler*.ab. OR toddler*.kw. OR toddler*.kf.) OR (newborn*.ti. OR newborn*.ab. OR newborn*.kw. OR newborn*.kf.) OR infant.sh. OR child.sh. OR child, preschool.sh. OR infant, newborn.sh.)
PubMed	(((((cow[Title/Abstract] OR cow's[Title/Abstract]) AND milk[Title/Abstract]) AND (allerg*[Title/Abstract] OR hypersensitiv*[Title/Abstract])) OR ((milk hypersensitivity[MeSH Terms]) OR (milk hypersensitivities[MeSH Terms]))) AND (((microb*[Title/Abstract]) OR (microflora[Title/Abstract]) OR (16S[Title/Abstract]) OR (bifido*[Title/Abstract]) OR (bacter*[Title/Abstract]) OR (lachno*[Title/Abstract]) OR (rumino*[Title/Abstract]) OR (veillo*[Title/Abstract]) OR (entero*[Title/Abstract])) OR ((microbiota[MeSH Terms]) OR (microbiotas[MeSH Terms]) OR (human microbiome[MeSH Terms]) OR (human microbiomes[MeSH Terms]) OR (microbiome[MeSH Terms]) OR (microbiome, human[MeSH Terms]) OR (microbiomes[MeSH Terms]) OR (16s ribosomal rna[MeSH Terms]) OR (ribosomal rna, 16s[MeSH Terms]) OR (rna, 16s ribosomal[MeSH Terms]) OR (bifidobacterium[MeSH Terms]) OR (bacteroidaceae[MeSH Terms]) OR (bacteroides[MeSH Terms])

	OR (ruminococcus[MeSH Terms]) OR (veillonellaceae[MeSH Terms]) OR (veillonella[MeSH Terms]) OR (enterobacteriaceae[MeSH Terms]))))) AND (((child*[Title/Abstract]) OR (infant*[Title/Abstract]) OR (baby[Title/Abstract]) OR (babies[Title/Abstract]) OR (toddler*[Title/Abstract]) OR (newborn*[Title/Abstract])) OR ((infant[MeSH Terms]) OR (child[MeSH Terms]) OR (child, preschool[MeSH Terms]) OR (infant, newborn[MeSH Terms]))))
Scopus	(TITLE-ABS-KEY (cow* W/6 milk)) AND ((TITLE-ABS-KEY (allergy)) OR (TITLE-ABS-KEY (hypersensitiv*))) AND ((TITLE-ABS-KEY (microb*)) OR (TITLE-ABS-KEY (microflora)) OR (TITLE-ABS-KEY (16s*)) OR (TITLE-ABS-KEY (bifido*)) OR (TITLE-ABS-KEY (bacter*)) OR (TITLE-ABS-KEY (lachno*)) OR (TITLE-ABS-KEY (rumino*)) OR (TITLE-ABS-KEY (veillo*)) OR (TITLE-ABS-KEY (entero*))) AND ((TITLE-ABS-KEY (child)) OR (TITLE-ABS-KEY (infant)) OR (TITLE-ABS-KEY (baby)) OR (TITLE-ABS-KEY (toddler)) OR (TITLE-ABS-KEY (newborn)))
Web of Science	(TI=(cow* AND milk) OR AB=(cow* AND milk) OR AK=(cow* AND milk) OR KP=(cow* AND milk)) AND ((TI=(allergy) OR AB=(allergy) OR AK=(allergy) OR KP=(allergy)) OR (TI=(hypersensitiv*) OR AB=(hypersensitiv*) OR AK=(hypersensitiv*) OR KP=(hypersensitiv*))) AND ((TI=(microb*) OR AB=(microb*) OR AK=(microb*) OR KP=(microb*)) OR (TI=(microflora) OR AB=(microflora) OR AK=(microflora) OR KP=(microflora)) OR (TI=(16s*) OR AB=(16s*) OR AK=(16s*) OR KP=(16s*)) OR (TI=(bifido*) OR AB=(bifido*) OR AK=(bifido*) OR KP=(bifido*)) OR (TI=(bacter*) OR AB=(bacter*) OR AK=(bacter*) OR KP=(bacter*)) OR (TI=(lachno*) OR AB=(lachno*) OR AK=(lachno*) OR KP=(lachno*)) OR (TI=(rumino*) OR AB=(rumino*) OR AK=(rumino*) OR KP=(rumino*)) OR (TI=(veillo*) OR AB=(veillo*) OR AK=(veillo*) OR KP=(veillo*)) OR (TI=(entero*) OR AB=(entero*) OR AK=(entero*) OR KP=(entero*))) AND ((TI=(child) OR AB=(child) OR AK=(child) OR KP=(child)) OR (TI=(infant) OR AB=(infant) OR AK=(infant) OR KP=(infant)) OR (TI=(baby) OR AB=(baby) OR AK=(baby) OR KP=(baby)) OR (TI=(toddler) OR AB=(toddler) OR AK=(toddler) OR KP=(toddler)) OR (TI=(newborn) OR AB=(newborn) OR AK=(newborn) OR KP=(newborn)))

Table S3. Information and reasons for the 28 papers excluded after careful consideration

Index	Author and year	Exclusion reason
1	Pohjavuori *et al.*, 2004[1]	Diagnosed IgE-mediated CMA based on a CM challenge and skin prick tests or antigen-specific IgE of any antigen tested (including also egg-white, cat, dog and birch).
2	Viljanen *et al*., 2005a[2]	
3	Barros *et al*., 2017[3]	Distinguished between IgE-mediated and non-IgE mediated CMA in the description of the allergic subjects but did not report any specific results for IgE-mediated CMA.
4	Viljanen *et al*., 2005b[4]	
5	Burks *et al*., 2015[5]	
6	Dong *et al*., 2018[6]	

Index	Author and year	Exclusion reason
7	Jarvinen *et al*., 2014[7]	Reported 29 infants with IgE-mediated CMA in their table with clinical characteristics. However, elevated levels of cow's milk specific IgE were reported in only 13 infants. The corresponding author was contacted by email, but was unable to supply additional data because the research was done in a previous institution
8	Mercer *et al*., 2009[8]	CMA was diagnosed based on total and CM specific IgE levels and CMA-related symptoms, but no oral food challenge was used to confirm CMA.
9	Taniuchi *et al*., 2005[9]	Included several subjects whose diagnosis was not confirmed by an oral food challenge, but by a cow's milk elimination diet
10	Kendler *et al*., 2006[10]	Did not confirm CMA by oral food challenge
11	Hol *et al*., 2008[11]	Used a food challenge, but diagnosed children based on their late response, which does not point to IgE-mediated CMA
12	Shek *et al*., 2005[12]	Included both children below 12 years old as well as adolescents and/or adults, but results for children were not reported separately
13	Yamamoto-Hanada *et al*., 2023[13]	
14	Hill *et al*., 1989[14]	Did not include any gut microbiome data or intervention targeting the gut microbiome
15	Hauer *et al*., 1997[15]	
16	Szabó and Eigenmann, 2000[16]	
17	Paparo *et al*., 2016[17]	Studied infants that received probiotics in the past, before entering the study, and therefore could not be compared to the probiotic intervention studies discussed in this review
18	Gotteland *et al*., 1992[18]	Studied CM protein absorption after *E. coli* infection
19	Morin *et al*., 2012[19]	Animals models were sensitized to CM, but did not receive a food challenge, thus focus on CM sensitization rather than CMA
20	Shandilya *et al*., 2016[20]	
21	Wróblewska *et al*., 2020[21]	
22	Maiga *et al*., 2017[22]	
23	Pescuma *et al*., 2019[23]	Two of the three experiments had no challenge, while in the third one there was no comparison between (allergy or treatment) groups
24	Graversen *et al*., 2021[24]	Focused on antibiotics instead of treatment for CMA.
25	Liu *et al*., 2023[25]	Studied the effect of pre-treatment with whey or beta-lactoglobulin (BLG) before sensitization
26	Mauras *et al*., 2019[26]	The CMA donor used for fecal transplantation had multiple food allergy
27	Schouten *et al.*,2009[27]	No GM-related data, do not mention how the treatment changed the GM
28	Adel-Patient *et al.*,2020[28]	

Table S4. CMA diagnosis and measured variables for all human studies. Abbreviations: see Table S1

Author and year	CMA diagnosis	Measured variables		
		Microbiome	**Metabolomics**	**Immune response**
Thompson-Chagoyan et al., 2010[29]	CM-specific IgE, SPT, DBPCFC	Aerobes, Anaerobes, Enterobacteria, Bifidobacteria, *Lactobacilli*, Clostridia	-	-
Salmi et al., 2010[30]	CM-specific IgE, SPT, DBPCFC	-	**Urine**: 37 organic acids, Creatinine	-
Thompson-Chagoyan et al., 2011[31]	CM-specific IgE, SPT, DBPCFC	10 targeted probes: *Bifidobacterium*, *Bacteroides,* Enterobacteria*, Streptococcus, Lactobacillus, Atopobium, Clostridium coccoides, Clostridium leptum, Clostridium perfringens sps., Clostridium difficile sps.*	**Feces**: Lactate, SCFA (acetate, propionate, butyrate, isocaproic acid), Branched-chain short fatty acids (BCSFA).	-
Francavilla et al., 2012[32]	CM-specific IgE, SPT, DBPCFC	13 targeted probes: Domain bacteria, negative control, *Bifidobacterium*, *Bacteroides/Prevotella*, Eubacterium rectale/Clostridium coccoides, *Lactobacillus/Enterococcus*, *Streptococcus/Lactococcus group*, *Escherichia coli*, Sulfate–reducing bacteria (SRB), *Atopobium* group, *Coriobacterium* group, *Clostridium histolyticum*, *Clostridium lituseburense*	**GC-MS (feces)**: 15 organic metabolites (esters, ketones, Alcohols, sulfur compounds, hydrocarbons, SCFA); **NMR (feces):** pyruvic acid, lactic acid, uridine, histidine, tyrosine, threonine, methionine, proline, TMAO, arginine/histidine, valine / isoleucine, phenylalanine, gamma–amino–butyric acid/lysine	-
Guo et al., 2016[33]	Analysis of serum samples, SPT, DBPCFC	Dominant bacteria, *Bifidobacterium, Lactobacillus, C. coccoides*, Microbiota diversity (Shannon-Weaver index, dice similarity coefficient)	-	-

Table S4. Continued

Author and year	CMA diagnosis	Measured variables		
		Microbiome	**Metabolomics**	**Immune response**
Canani et al., 2016[34]	Clinical history, CM-specific IgE, DBPCFC	Dominant bacteria, Microbiota Alpha diversity (Shannon index) and Evenness (Pielou's evenness index)	**Feces**: butyrate	-
Dong et al., 2018	CM-specific IgE, SPT, DBPCFC	Dominant bacteria, Microbiota Alpha diversity (Chao1, ACE, Simpson, Shannon, and coverage indices)	**Feces**: SCFAs (acetate, butyrate, propionate, isobutyrate), lactate	-
Mennini et al., 2021[36]	CM-specific IgE, SPT, DBPCFC	PCR : Dominant bacteria; qRT-PCR: *B. breve, B. longum subsp. longum, B. longum subsp. infantis* Microbiota Alpha diversity (Observed, Chao1 and Shannon indices) and beta diversity(unweighted UniFrac)	-	-
Mera-Berriatua et al., 2022[37]	Clinical history of IgE-mediated food allergy, SPT	Dominant bacteria Microbiota Alpha diversity (Shannon index) and beta diversity (Bray-Curtis distance)	-	-
Bunyavanich et al., 2016[38]	CM-specific IgE, SPT, CM challenge or AD with CM-specific IgE	Microbiome (feces): Dominant bacteria; Microbiota Alpha diversity (Faith's phylogenetic diversity) and beta diversity (unweighted UniFrac)	-	-

Table S4. Continued

Author and year	CMA diagnosis	Measured variables		
		Microbiome	**Metabolomics**	**Immune response**
Dupont et al., 2015[39]	CM-specific IgE, SPT, or both positive cutaneous tests and IgE, DBPCFC	Total bacteria, *Clostridium* cluster IV, *Bacteroides/ Prevotella* group, *Bifidobacterium, Lactobacillus/ Leuconostoc/Pediococcus* group, *Clostridium* cluster XIVa, *Clostridium* cluster XI, *Clostridium* cluster I/II, *Staphylococcus, Enterococcus, Escherichia coli*	**Plasma:** Amino acids (cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, valine) **Feces:** butyrate	-
Chatchatee et al., 2022[40]	CM-specific IgE, SPT, DBPCFC	bifidobacteria and ER/CC group	-	-
Jing et al. 2020[41]	SPT, IgE, DBPCFC	dominant bacteria microbiota Alpha diversity (number of OTUs, Chao1, Shannon, Simpson index) and beta diversity (weighted and unweighted UniFrac)	-	**Immunoglobulins** Total IgE, IgG_2 (serum) **Cytokines** TNFα, IL-1β, IL-6, IL-10 (serum)

Table S5. Model information for all animal studies. Abbreviations: see Table S1

Sensitization					Challenge		Intervention details		n size/ group † †	Author and year
Animal/ Strain (gender)	**Allergen: Dose(mg)**	**Adjuvant: Dose (µg)**	**Period† (wk)**	**Admini-stration**	**Intradermal Allergen dose (ug)**	**Intragastric Allergen dose(mg)**	**Intro-duction**	**Duration**		
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:20	W:50	Pre-S	6-9d‡	6-8	Kostadinova et al.2017a[42]
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:20	W:50	Pre-S	6-9d‡	6-8	Kostadinova et al 2017b.[43]
C3H/HeOuJ mice (F)	W:20	CT:10	5	i.g.	W:6	W:50	**Long:** WS **Short:** Pre-S	**Long:** 7.5wk **Short:** 5d	7-10	Kleinjans et al.2019[44]
BALB/cByJ mice (F)	W:15	CT:10	5	i.g.	-	BLG:60	WS	6wk	30	Neau et al.,2016[45]
BALB/cByJ mice (F)	W:15	CT:10	5	i.g.	-	BLG:60	Post-S	20d	10 -12	Esber et al.2020[46]
Germ-free C3H/HeN	BLG:20	CT:10	5	i.g.	-	BLG:2*100	-	-	6-42	Feehley et al.2019[47]
C3H/HeN mice (M)	W/W/W: 10/100/0.5	CT/CT/Alum: 10/10/2	5/2/2	i.g./i.g./i.p.	-	W:50	-	-	3-7	Cao et al. 2022[48]
C57BL/6J and BALB/cJ (M and F)	BLG:1	CT:10	5	i.g.	-	W:50	-	-	5-10	Smith et al. 2021[49]

†All administrations are performed weekly
†† Intervention group sizes (not control group)
‡ Synbiotic diet for 9 days, peptide mix intake for 6 days

Table S6. Measured variables for all animal studies. Abbreviations: see Table S1

Author and year	Measured variables		
	Microbiome	**Metabolomics**	**Immune response**
Neau et al.[45]	11 bacteria primers, all bacteria	-	**Igs:** Total and BLG-s IgE, IgG_1, IgG_{2a} (plasma) **Cytokines:** IFN-γ, IL-12p70, IL-4, IL-5, and IL-10 (spleen, MLN) **mRNA expression:** ifn-g, il-4, il-10, tgf-b, il-17a, t-bet, gata3, rorγt, foxp3 (ileum)
Esber et al.[46]	α (Shannon index) and β (Bray-Curtis distance, UniFrac distance) diversity	**Feces**: SCFA, **Plasma**: other metabolites	**Igs:** BLG- sIgE, $sIgG_1$, $sIgG_2$ (plasma) **Cytokines:** IL-17A, IL-2, GM-CSF, IL-4, IFN-γ, IL-10, IL-5, IL-12p70 (spleen) **mRNA expression:** gata3, tbet, foxp3, rorγt, ifnγ, tnfα, il4, Il10, and tgfβ (ileal)
Kleinjans et al.[44]	All bacteria	-	**Igs:** W- sIgE, $sIgG_1$, $sIgG_{2a}$ (serum)
Kostadinova et al.[42]	-	**Feces**: acetic acid, propionic acid, butyric acid	**Igs:** W- and BLG- sIgE, $sIgG_1$, $sIgG_{2a}$ (serum) **Lymphocytes:** T cells, DC (spleen, MLN, SI-LP) **Cytokines:** IL-5, IL-13, IL-10, IL-17A, IFN-γ (Spleen, MLN, SILP)
Kostadinova et al.[43]	-	<u>Part 1: Post-oral tolerance</u> **Metabolites** **Feces**: acetic acid, propionic acid, butyric acid, valeric acid	<u>Part 1: Post-oral tolerance</u> **mRNA expression:** Foxp3, Tbet, GATA3, RorγT, IL-10, galectin-9, TGF-β, IL-13, IFN-γ, IL-22 (PP, SI (proximal, middle), colon) **Immunohistochemistry**: Foxp3+ cells (colon) <u>Part 2: Post-challenge</u> **mRNA expression:** Foxp3, Tbet, GATA3, RorγT, IL-10, galectin-9, TGF-β, IL-13, IFN-γ, and IL-22 (PP, spleen) **Lymphocytes:** T_{reg} (LP)
Smith et al.[24]	α (Shannon, Simpson indices) and β(Bray-Curtis) diversity	-	**Igs:** BLG-sIgE,s sIgG1; sIgG2a (serum) **Cytokines, chemokines, and acute phase proteins:** e.g. IL-10, IL-13, IL-15, IL-1β, IL-31, IL-21, CCL1, CCL9, CCL12, CCL17, FGF2, CDF1, CSF2, TNFFSF1A, TNFRSF1B, ICAM-1 (plasma)

Table S6. Continued

Author and year	Measured variables		
	Microbiome	**Metabolomics**	**Immune response**
Cao et al.[23]	All bacteria, α and β diversity	-	**Igs:** W- sIgE, $sIgG_1$, $sIgG_{2a}$ (serum) **Cytokines:** IL-6, IL-10 (serum) **mRNA expression:** IL-4, IL-8, IL-33, IL-1β, TGF-β, GAPDH, mTOR mRNA
Feehley et al.[47]	α (Shannon index) and β (weighted UniFrac) diversity Pielous' s evenness	-	**Igs:** BLG-specific IgE, IgG_1 (serum) **Cytokines:** IL-13, IL-4 (spleen) ex-W **Transcriptome:** 32 genes (IEC)

Supplementary References

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