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Immunoglobulin-coating patterns reveal altered humoral responses to gut bacteria in pediatric cow milk allergies

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Abstract

Background Pediatric cow milk allergies (CMA) can occur in immunoglobulin (Ig) E and non-IgE-mediated forms. Unlike IgE-mediated allergies, the mechanisms of disease pathogenesis in non-IgE-mediated food allergy and an association with microbiome has not been well established. Previous studies have identified the presence of altered humoral responses to gut bacteria in IgE mediated allergies. Here, we analyzed IgA, IgE and IgG responses to gut bacteria in subjects with either IgE or non-IgE mediated CMA to identify relative proportions of Ig-coated bacteria and characterize unique disease specific microbial signatures.

Methods Multi-parametric flow cytometry analysis was used to identify IgA, IgE and IgG responses to gut bacteria in CMA patients. Cell sorting of Ig coated gut bacteria was subsequently performed followed by high throughput 16S rRNA gene sequencing and specific patterns of humoral responses to gut bacteria assessed in each study group.

Results We identified significant alterations in IgA and IgG gut bacterial coating patterns in CMA subjects. Proportions of IgA-coated bacteria were decreased in IgE mediated CMA subjects without atopic dermatitis (ALL) and non-IgE mediated CMA subjects (ENP), compared to healthy controls (CON). In comparison, IgG-coated bacteria was significantly elevated in CMA subjects with atopic dermatitis (AD). Alpha and beta diversities displayed significant differences in IgA-, IgE-, and IgG-coated bacteria in AD and ENP groups. Significant differences in bacteria coated by IgA, IgE and IgG were detected at Phyla, Genus and Species levels and associated bacterial dysbiosis in IgE and non-IgE mediated allergies were identified. Linear discriminant analysis (LDA) effect size (LEFse) revealed unique disease associated bacterial signatures, including several pathogenic bacteria namely *Bacteroides fragilis*, *Ruminococcus gnavus*, *Eubacterium dolichum*, *Fusobacterium*, *Clostridium neonatale* and *Robinsoniella peoriensis*. Receiver operating characteristic curve analysis confirmed the efficiency of using the bacterial signatures identified as biomarkers for disease.

Conclusions Altered IgA and IgG responses to gut bacteria were identified in CMA subjects. The disease-specific responses were associated with alterations in bacterial diversity and concomitant dysbiosis of Ig-coated bacteria in IgE-mediated and non-IgE-mediated CMA pediatric subjects. The identification of pathogenic bacteria uniquely associated with different classes of allergic disease indicates a role of these bacteria in driving disease-specific pathological phenotypes.

Keywords IgA, IgE, IgG, Gut bacteria, Immunoglobulin-coating, Cow milk allergy, Microbial dysbiosis

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Background

Cow milk allergy (CMA) is the most prevalent food allergy amongst young children, manifesting as either an IgE mediated or non-IgE mediated allergic response [1]. CMA typically presents within the first year of life and amongst infants at one year of age, rates of CMA have been estimated to be between 0.5% and 3% [2], with the occurrence of CMA showing significant increases over the past three decades [1]. Studies looking at CMA resolution have shown a variety of outcomes, with one study showing 47% resolution by age 4 to 5 [3], whereas others have noted a continued prevalence of 57% CMA reactive children at age 10 [4]. In comparison, the incidence of CMA in the adult population is considerably lower with a recent study reporting a rate of 1.9%, of which 77.3% of these adults had first experienced CMA during early childhood [5]. Previous studies have shown that dysbiosis in the gut microbiome plays a significant role in the development of pediatric CMA [6, 7], as the gut microbiota has a pivotal role in the maintenance of homeostasis and in regulating immune responses at the intestinal barrier [8]. Several factors, including the mode of delivery [9], use of antibiotics [10] and breast feeding versus formula feeding [11–13], can contribute to dysbiosis of the gut microbiota, which in turn, leads to the predisposition of the development of atopic disease [14]. Reduced prenatal and postnatal microbial exposure is associated with a predisposition to the development of IgE mediated allergic diseases [15–18]. Under homeostatic conditions, the gut microbiota forms a symbiotic relationship with the host through reciprocal interactions between the intestinal mucosal surface, and the host immune system [19]. The development of a diverse gut microbiome helps in shaping immune tolerance to symbiotic bacteria and allows both the induction of protective responses against pathogens and maintenance of regulatory pathways [20, 21]. The presence of a diverse microbiome results in the immune system becoming tolerized to innocuous antigens from commensal bacteria and potential food allergens, through the induction of an IgA mediated antibody response, in association with a corresponding complement of regulatory T cells [14]. However, perturbations to the composition of the microbiome can cause microbial dysbiosis, leading to abnormal immune responses, inflammation and allergic diseases [20, 22].

In contrast to IgE mediated allergic diseases, the mechanisms associated with disease pathogenesis in non-IgE mediated food allergy are not yet well described and a link with microbial dysbiosis has not been definitively established [23, 24]. A previous study on non-IgE mediated CMA identified overlapping signatures in the dysbiosis exhibited by IgE and non-IgE

mediated CMA children [25] and a second recent study identified a significant decrease in fecal *Bifidobacterium* to be a potential biomarker associated with onset of non-IgE mediated CMA. In general, non-IgE-mediated food allergies primarily affect the gastrointestinal system and are characterized by enteropathy and colitis-based symptoms in disorders including; food protein-induced allergic enteropathy (FPE), food protein-induced enterocolitis syndrome (FPIES) and food protein-induced allergic proctocolitis (FPIAP) [26]. Non-IgE mediated allergies are thought to be mediated by cell driven adaptive and innate immune mechanism [27] based on the delayed onset of symptoms following ingestion of allergens in combination with the absence of an IgE mediated response [28].

The immunoglobulins IgA, IgG and IgE are produced as a result of class switching events following activation and differentiation of naive IgM-bearing B cells, in response to an antigenic or allergenic stimulation [29]. While IgA is predominantly secreted across mucosal barriers and in breast milk and functions as an immune modulator at mucosal surfaces, IgG antibodies are most highly abundant in lymph and blood and offer broad systemic protection [29]. IgE is found at the lowest concentration, due to the tight regulation of IgE class switching events and sequestration at cell surfaces via binding to high and low FcE receptors [29]. Although class switching in favor of IgE occurs in the nasal [30] and bronchial mucosa [31] of allergic patients, the specific sites and role of IgE production in the gastrointestinal tract are still poorly characterized [29].

Previous studies have shown that IgA and IgG antibodies can bind to fecal microbes in murine samples, as well as in patient samples with inflammatory bowel disease (IBD) [32, 33]. In the context of IBD, IgG coating was observed mainly in subjects with IBD and was largely absent in healthy controls [34]. Studies have shown that IgA secreted at mucosal surfaces coats a fraction of the intestinal microbiota through both T-cell dependent and independent pathways, where the alternate pathways were shown to produce IgA that bound distinct subsets of microbes [35]. Further, IgA has been shown to maintain the composition of the microbiota and decreased concentrations of intestinal IgA have been correlated with lower levels of bacterial diversity [36] and high levels of intestinal IgA have been associated with a reduced risk of developing IgE mediated allergic disease [37]. Further, a reduction in levels of IgA coated bacteria and an increase in IgE coated bacteria was observed in food allergic patients [38]. Food allergy has also been associated with increased anti-commensal IgE-coating in the presence of *Akkermansia muciniphila* in fiber-deprived mice [39].

The association of Ig-coated bacteria (IgA, IgE and IgG) with disease has not previously been studied in the context of IgE vs. non-IgE mediated allergic disease. In this study, we recruited children with both IgE and non-IgE mediated CMA and identified the presence of differential patterns of bacterial Ig coating amongst disease groups, leading to the identification of altered Ig-coated microbial signatures in CMA subjects, which uniquely distinguished the specific allergic groups studied.

Methods

Ethics approval and consent to participate

The study obtained written parental consent from all participants before enrollment. The study protocol (1500751) was approved by the Institutional Review Board (IRB) at Sidra Medicine, Doha, Qatar.

Study design and patient recruitment

Recruitment of study subjects was conducted following Institutional Review board (Sidra Medicine, Doha, Qatar) approval, #1706011686. Informed consent was obtained from the parents/guardians of the study subjects. Pediatric subjects, aged between 1 and 4 years, with clinical symptoms of CMA, were recruited to the study and were grouped into IgE and non-IgE mediated allergic groups based on the clinical manifestations and the time of onset allergic symptoms. Age-matched healthy subjects served as the control group. A total of 38 study subjects were recruited and categorized into four groups; Control population with no history of food allergies (CON=13), IgE mediated CMA (ALL=8), IgE mediated CMA with

Atopic Dermatitis (AD=10), non-IgE mediated CMA with Enteropathy (ENP=7), all patient characteristics are included in Table 1. Study exclusion criteria were, use of oral steroids, recent use of antibiotics, atopic dermatitis without sensitivity to milk, early onset inflammatory bowel diseases not related to milk ingestion, celiac disease and chronic diarrhea due to other immune defects. From all the study subjects, data was collected from a questionnaire which provided information regarding the familial history of allergy and environmental exposures.

ImmunoCAPS assay for total and cow-milk specific IgE

Serum measurements of total IgE levels and total cow milk protein, casein-specific, β -lactoglobulin specific and α -lactalbumin specific IgE (sIgE), were performed using a fluoroenzyme immunoassay auto-analyzer, the ImmunoCAP 100 platform (ThermoFisher), according to the manufacturer's guidelines [40] at the Hematology, Immunology and Transfusion Division, Sidra Medicine, Doha, Qatar.

Stool sample collection

Stool samples were collected in collection tubes and were frozen immediately at -20°C after collection and subsequently stored at -80°C until processing. The frozen stool samples were thawed immediately before the bacterial isolation.

Bacterial isolation, flow cytometry and sorting

Frozen stool samples were thawed on ice and 100 mg of stool sample was homogenized in 1X DPBS at a

Table 1 Demographics of study subjects

Patient demographics	CON	ALL	AD	ENP	p ALL vs. CON	p AD vs. CON	p ENP vs. CON
N	13	8	10	7	N/A	N/A	N/A
Male (%) ^a	38	75	60	43	ns	ns	ns
Caesarean delivery (%) ^a	38	25	50	29	ns	ns	ns
Gestational age, weeks (mean, SD) ^o	37.92, 2.10	38.88, 1.13	39.10, 1.97	39.14, 0.69	ns	ns	ns
Birth weight, kg (mean, SD) ^o	2.76, 0.56	2.88, 0.45	3.25, 0.45	3.23, 0.69	ns	ns	ns
Age of Onset, months (mean, SD) ^o	N/A	5.12, 3.39	4.2, 2.6	3.85, 4.4	N/A	N/A	N/A
Breast Feeding 0–6 months (%) ^a	92.31	100.00	90.00	100.00	ns	ns	ns
Formula Feeding 0–6 months (%) ^a	30.70	12.50	40.00	28.50	ns	ns	ns
Solid Food Introduction, months (mean, SD) ^o	5.46, 1.71	5.87, 0.35	6.9, 1.91	7.28, 4.78	ns	ns	ns
Playgroup at 6 months (%) ^a	7.69	12.50	10.00	28.57	ns	ns	ns
Maternal antibiotic use during pregnancy (%) ^a	30.77	50.00	10.00	14.29	ns	ns	ns
Exposure to cigarette smoke at home (%) ^a	15.38	12.50	0.00	0.00	ns	ns	ns
Vitamin D deficiency at birth (%) ^a	76.92	87.50	70.00	85.71	ns	ns	ns
Direct familial allergy history-parents/siblings (%) ^a	15.38	50.00	80.00	71.43	ns	**	*
Indirect familial allergy history-grandparents (%) ^a	0.00	37.50	40.00	0.00	*	*	ns

^aChi square used as statistical test

^oKruskal Wallis with Dunn's multiple comparison used as statistical test

concentration of 100 mg/ml and centrifuged at 50 g for 15 min at 4 °C to remove large particles in the stool. Supernatants were recovered and washed twice at 8000 g for 5 min at 4 °C with 1 ml 1X DPBS. Following an additional wash, bacterial pellets were resuspended and incubated for 20 min at 4 °C in 1X DPBS containing 20% mouse serum (Sigma-Aldrich, Cat# M5905). Bacteria were then stained in a mixture of 1:20 IgA-APC (Clone: IS11-8E10, Miltenyi Biotec, Cat# 130-113-472), 1:20 IgE-PE (Clone: MB10-5C4, Miltenyi Biotec, Cat# 130-119-899) and 1:20 IgG-PerCP Vio700 (Clone: IS11-3B2.2.3, Miltenyi Biotec, Cat# 130-119-880) and Hoechst 33342 (10 µg/ml, BD Pharmingen, Cat# 561908) for 1 h on ice. Unstained and Hoescht only controls for each sample were also prepared. The samples were then washed in FACS buffer (1X DPBS + 1%FBS + 2 Mm EDTA) and acquired on BD FACS Aria III. IgA, IgE and IgG single positive samples were acquired based on gating established from unstained and Hoescht only samples, in addition to Fluorescent Minus One (FMO) controls for each antibody. All samples were subsequently analyzed using FlowJo (Treestar) software.

Bacterial DNA extraction

Total DNA from the sorted mono-Ig coated bacterial samples was extracted using QIAamp Fast DNA stool Mini Kit (QIAGEN, Cat# 51604), according to manufacturer's instructions. The extracted DNA were stored at - 20 °C. The DNA integrity and concentration was measured using Qubit™ dsDNA Quantitation High Sensitivity Assay kit before the sequencing was performed.

16S rRNA gene amplification and sequencing

From the total isolated DNA, the V3–V4 regions of the 16S rRNA gene was amplified using Illumina Nextera XT library preparation Kit (Illumina, Cat# FC-131-1002), and specific primers for V3-V4 region amplification:

Forward 5' TCGTCGGCAGCGTCAGATGTGTAT
AAGAGACAGCCTACGGGNGGCWGCAG
Reverse 5' GTCTCGTGGGCTCGGAGATGTGTA
TAAGAGACAGGACTACHVGGGTATCTAATCC

PCR amplification was done using equal starting DNA concentration. The amplified PCR products were purified using the Illumina 16S metagenomic sequencing library preparation protocol as per the instructions using AMPure XP magnetic beads (Beckman Coulter, Cat# A63881). The dual Index PCR was performed using the same protocol and purified as per the instructions. High-throughput sequencing was performed on an Illumina MiSeq2×300 platform in accordance with the manufacturer's instructions.

Sequence analysis and taxonomic identification

The sequenced data was demultiplexed using the MiSeq Control Software (MCS), processed, and analyzed as described in the previous work using QIIME v1.9.0 pipeline [41]. Operational taxonomic units (OTUs) were generated by aligning against the Greengenes database (Version 13_8) with a confidence threshold of 97% [42]. Alpha diversity measures (Chao1, Observed, Shannon and Simpson indices) were calculated using "Phyloseq", an R package [43]. Beta diversity indices were shown as principal coordinate analysis and R package "MicroEco" was used to perform the differences in the Bray–Curtis distance matrix between the study groups [44]. Differentially abundant taxa between the studied groups were identified using Linear Discriminant Analysis Effect Size (LEfSe) tool [45]. Microbial dysbiosis was measured using the package "dysbiosisR" and shared OTUs/taxa between the study groups were performed using the packages "MicroEco" and "Microbiota Process" [46, 47].

Statistical analysis

Statistical tests were performed using GraphPad PRISM software 10.2.3 (GraphPad Software, La Jolla, CA, USA) and data are shown as mean ± SEM. Shapiro-Wilks normality analysis was used to determine whether the data was normally distributed. Quantitative variables were compared using Kruskal Wallis-Dunn's post-hoc analysis, one-way or two-way ANOVA-Tukey post-hoc analysis and one-way ANOVA-Dunnet post-hoc analysis as indicated for each figure panel. Statistical significance is defined as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. Data are represented as mean ± SEM.

Results

Altered IgA coating within gut bacterial populations in IgE and non-IgE mediated cow milk allergic pediatric subjects

To study the differential immunoglobulin responses generated against gut resident bacteria in allergic subjects, we utilised a flow cytometry-based antibody detection assay to allow for the identification and multiparametric analysis of Ig bound bacteria and subsequent sorting. Using multicolor flow cytometry, we performed parallel phenotyping of IgA, IgE and IgG coated bacteria in control (n = 13) and CMA subjects (n = 25). Allergic subjects were grouped into IgE mediated CMA without atopic dermatitis (ALL = 8), IgE mediated CMA with atopic dermatitis (AD = 10) and non-IgE mediated CMA with enteropathy (ENP = 7) groups (Fig. 1A). The demographics of the study subjects are detailed in Table 1. There were no significant differences in the gender, mode of delivery, gestational age, breastfeeding/formula feeding, solid food introduction, playgroup, maternal use of

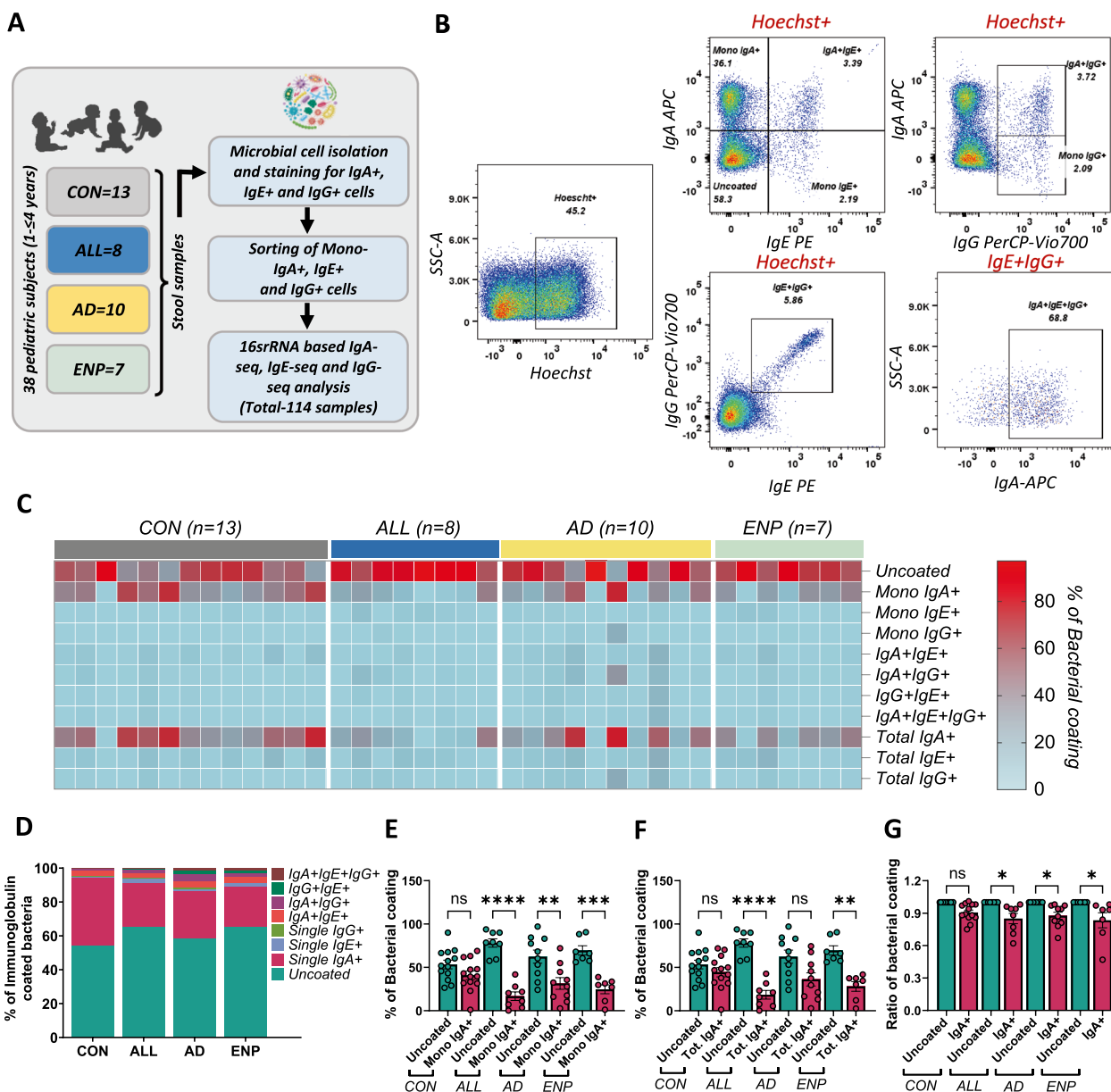


Fig. 1 Altered IgA coating in cow milk allergic subjects. **A** Study outline. **B** Gating strategy used for identifying bacterial coating in the study subjects. **C** Heat-map showing the differential coating of bacterial populations in the study subjects. **D** Stacked plot for differential bacterial coating among the study groups. **E** and **F** Quantitative analysis of differential distribution of IgA + (**E**) and total IgA + (**F**) alongside uncoated bacteria within the study groups. **G** Quantitative analysis of the ratio of mono-IgA+ to total IgA+ coated bacteria compared to the normalised level of uncoated bacteria within the study groups. Quantitative variables were compared using one-way ANOVA and Tukey post-hoc analysis. Statistical significance is defined as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Data are represented as mean \pm SEM. See also Supplementary Figure S1 for fluorescence minus one and background staining controls

antibiotics and exposure to cigarette smoke, between the controls and those infants with allergic manifestations (Table 1). However, significant differences in both direct and indirect familial histories of allergy were detected (Table 1). ImmunoCAPs testing was conducted to determine levels of total IgE, total cow milk protein-, casein-, β -lactoglobulin- and α -lactalbumin-specific-IgE (sIgE).

Our data showed increased levels of IgE and sIgE in the IgE mediated CMA groups (ALL and AD), whereas basal levels were detected in both CON and ENP groups (Table 2).

IgA, IgE, and IgG-coated bacterial cells extracted from fecal samples, were analyzed by flow cytometry according to the gating strategy outlined (Fig. 1B and

Table 2 Comparative analysis of plasma IgE levels in study subjects for total and cow-milk allergen-specific IgE

Plasma IgE levels (kUAD/L)	CON	ALL	AD	ENP	p ALL vs. CON	p AD vs. CON	p ENP vs. CON	p ALL vs. AD	p ALL vs. ENP	p AD vs. ENP
IgE Total (mean, SD) ^o	24.38, 24.75	117.8, 99.51	956.4, 1101	17.26, 14.6	ns	**	ns	ns	*	**
sigE CMP (F2) (mean, SD) ^o	0.14, 0.12	9.18, 8.95	111.6, 270.4	0.21, 0.35	*	**	ns	ns	**	**
sigE Casein (F78) (mean, SD) ^o	0.08, 0.07	6.55, 9.16	102.2, 255.4	0.08, 0.05	*	*	ns	ns	ns	ns
sigE β-lactoglobulin (F77) (mean, SD) ^o	0.06, 0.06	2.13, 2.28	8.17, 11.91	0.06, 0.08	*	**	ns	ns	*	**
sigE α-lactalbumin (F76) (mean, SD) ^o	0.06, 0.09	3.01, 2.02	5.84, 7.26	0.14, 0.33	*	ns	ns	ns	**	*

^oKruskalWallis with Dunn's multiple comparison used as statistical test

Supplementary Figure S1). Initial examination of the bacterial Ig coating revealed that a substantial proportion of bacteria remained uncoated, with no IgA, IgE or IgG binding detected (Fig. 1C and D). Amongst the Ig-coated bacteria, we identified a predominance of IgA coating across all subject groups (Fig. 1C and D). In contrast, IgE and IgG coating were observed at lower levels in all four study groups (Fig. 1C and D). We identified that Ig coating of bacteria occurred in multiple configurations as single, double and triple positive bacteria were observed across the study populations. Single- and double-coated bacteria were generally observed at higher frequencies, whereas triple-coated bacteria were observed at the lowest frequency and were absent in a proportion of the samples (Fig. 1C and D).

Previous studies have identified the association of reduced fecal IgA with food sensitization [48] and a decreased level of IgA coated bacteria in individuals with allergic conditions [21]. To investigate the patterns of overall IgA coating within our CMA study groups, we compared the percentages of mono-coated IgA+ (Fig. 1E) and total IgA+ (Fig. 1F) bacteria against uncoated bacteria. Here, we observed a decrease in the proportion of bacteria bound by IgA compared to uncoated bacteria present, with significant reduction in the mono-IgA+ coated bacteria in all the three allergic disease states (Fig. 1E), while the total IgA+ showed reduction of IgA+ coated bacteria in ALL and ENP groups (Fig. 1F). In contrast, we observed levels of both mono-IgA+ coated and total IgA+ coated bacteria to be similar to those of uncoated bacteria in our control group (Fig. 1E and F), potentially indicating the presence of an altered IgA bound microbial abundance and composition amongst the CMA groups. To further assess changes in differential IgA coating, we quantified the fraction of mono-IgA+ to total IgA+ coated bacteria and compared the proportion of bacterial IgA coating against the normalized levels of uncoated bacterial proportions in each study group. Here, we identified that the bacterial IgA coating significantly decreased in ALL, AD and ENP allergy groups, while there was no significant differences in the CON group (Fig. 1G), further indicating that, a reduction of bacterial IgA coating occurs in both IgE and non-IgE mediated pediatric CMA subjects.

Differential patterns of Ig coating in CMA subjects

To identify the differential patterns of Ig coating present among the study groups, we quantified and compared the levels unbound and bound bacteria across the study groups. On analyzing the proportions of mono-IgA, IgE and IgG-coated bacteria, we identified a decrease in the percentages of IgA coating in all allergic groups with a significant decrease observed in the ALL group and the ENP group as compared with controls (Fig. 2A). Although reported by previous studies for IgE mediated allergic disease [21, 38], a decrease in bacterial IgA coating in a non-IgE-mediated allergic disease has not been previously identified. Although we observed variations in mono-IgE and mono-IgG coated bacteria, this did not yield a statistically significant result (Fig. 2B and C). Patterns of total IgA-coated bacteria showed decreased percentages in all allergy groups, with a significant decrease of total IgA-bound bacteria detected in the ALL and ENP groups (Fig. 2D). We also observed a significant increase in the total IgG bound bacteria in the AD group (Fig. 2F). However, levels of total IgE-coated bacteria, did not show any significant differences between the control and allergic subjects (Fig. 2E). Analysis of the proportion of uncoated bacteria revealed an increase in the percentage of unbound bacteria in allergic subjects, but only the ALL group showed a significant increase as compared to controls (Fig. 2G).

Further, we analyzed the ratios of mono and total IgA, IgE and IgG-coated bacteria to uncoated bacteria present (Fig. 2H–M). We observed a significant decrease in mono and total IgA-coated bacteria in ALL and ENP groups (Fig. 2H and K), and a significant increase in the ratio of total IgG-bound bacteria in the AD group (Fig. 2M). Next, we looked at the proportions of IgA+IgE+, IgA+IgG+, IgE+IgG+ and IgA+IgE+IgG+ coated bacteria present (Figs. 2N and Q). Interestingly, we identified a significant increase in the proportion of dual bound IgA+IgG+ bound bacteria in the AD group compared to the CON group (Fig. 2O). Together, these findings indicate the presence of significant alterations in the proportions of Ig-coated bacteria in the IgE and non-IgE mediated CMA groups. Additionally, no increases in levels of IgE-coated

(See figure on next page.)

Fig. 2 Differential bacterial coating of IgA, IgE and IgG in cow milk allergic subjects. **A, B** Comparative analysis of mono-Ig coated and total Ig coated bacterial populations with respect to control subjects. **C** Quantification of uncoated bacteria across the study subjects. **D** and **E** Comparative analysis of the ratio of mono-Ig coated (**D**) and total Ig coated (**E**) bacteria to uncoated bacteria in the study subjects. **F** Comparison analysis of dual IgA+IgE+, IgA+IgG+ and IgE+IgG+ coated bacteria across the study groups. **F** Quantification of triple coated (IgA+IgE+IgG+) bacteria across the study subjects. Quantitative variables were compared using one-way ANOVA and Dunnett post-hoc analysis. Statistical significance is defined as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Data are represented as mean \pm SEM

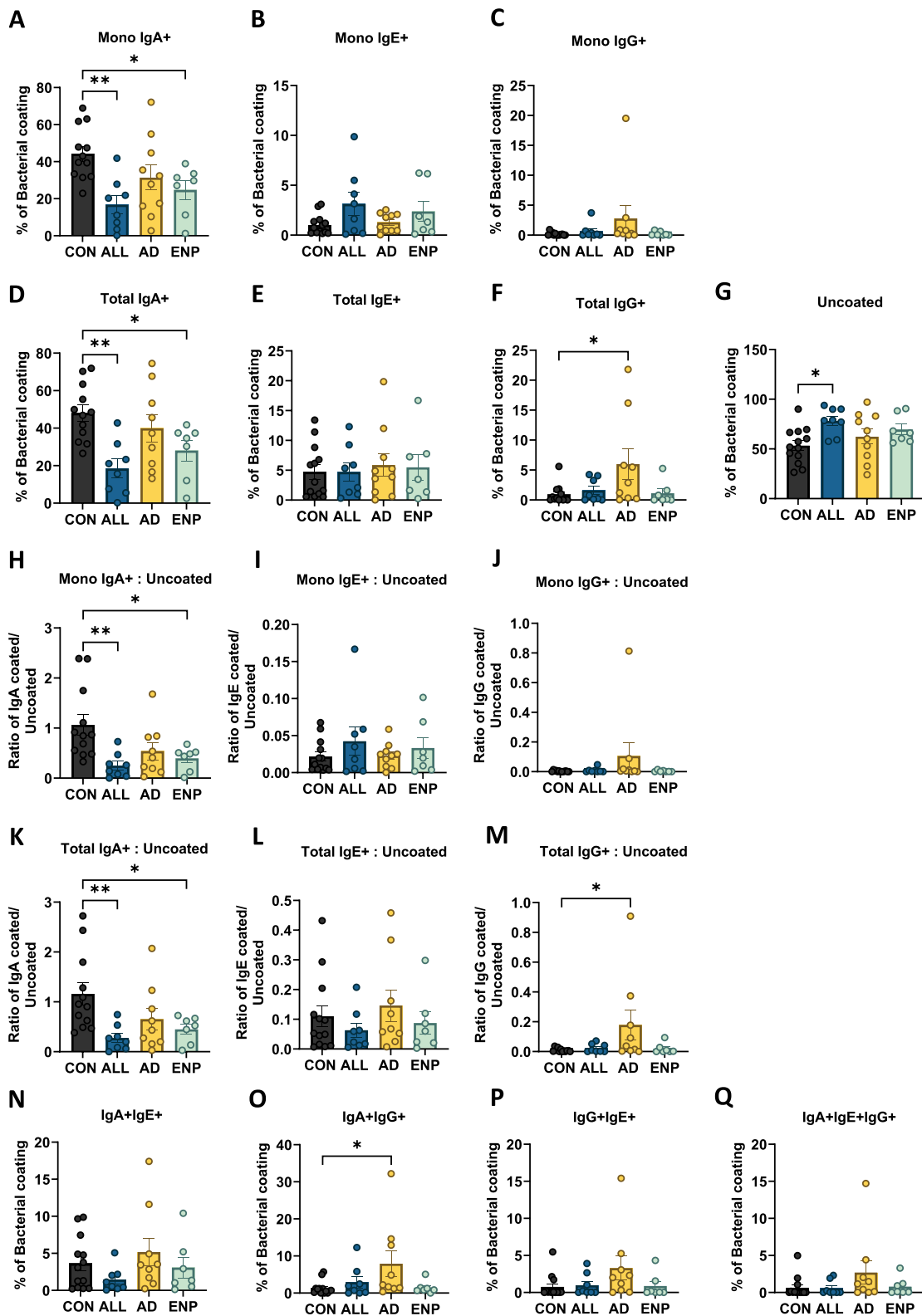


Fig. 2 (See legend on previous page.)

bacteria were detected in the subjects with IgE-mediated disease, despite the presence of significantly higher levels of IgE in their plasma (Table 2).

Immunoglobulin coating reveals alterations in levels of coated fecal bacterial OTUs

To identify whether unique Ig coated bacterial profiles are associated with the different disease groups, we sorted bacterial populations that were mono-IgA, mono-IgE or mono-IgG-coated and performed 16S rRNA gene sequencing. After quality control, a total of 9,955,536 sequences (median: 102,614) were used for OTU generation. Following filtering and alignment, a total of 8126 OTUs were generated, with an average of 87,329 reads per sample.

We initially examined the patterns of unique and common OTUs identified as being bound by IgA, IgE or IgG (Fig. 3A–D). In CON, ALL and AD patients. Here, the relative Ig-recognized OTU set size present, indicated a pattern of Ig recognition where IgA > IgE > IgG (Fig. 3A–C), whereas for ENP patients, the set size pattern showed IgE > IgA > IgG (Fig. 3D). Interestingly, in all subject groups, we identified bacterial OTUs that were recognized by more than one of each of the mono-Ig subsets studied. Here, these shared OTUs recognized all three Ig subsets and constituted the largest OTU groups observed in all four of the study groups (Fig. 3A–D). This indicated that different bacterial OTUs belonging to the same taxonomic level could be individually coated by IgA, IgE and IgG.

The OTUs recognized individually by any two Ig subsets displayed a pattern where OTUs common to IgA + IgE + > IgA + IgG + > IgE + IgG + in CON, ALL and AD, whereas in the ENP group, the pattern observed was IgA + IgE + > IgE + IgG + > IgA + IgG + (Fig. 3A–D).

Further, as an estimate of the Ig-bound repertoire diversity present for each study subset, we first analyzed the total OTU counts in each study group for IgA, IgE and IgG subsets and identified a significantly lower number of IgA bound OTUs in the ENP group as compared to the CON group (Fig. 3E). We then used the number of OTUs present in individual study subjects' samples to calculate the comparative proportion of bacteria bound by unique Ig's as determined by flow cytometry to

generate an Ig repertoire diversity index for IgA, IgE and IgG. This analysis showed a significant decrease in the repertoire diversity index for the IgA subset in all three CMA groups (Fig. 3F). No significant differences were observed in the diversity index calculated among the study groups for the IgE and IgG bound subsets (Fig. 3F).

Loss of alpha diversity and altered beta diversity in cow milk allergic subjects

The alpha diversity of the Ig coated bacteria was assessed to determine the species richness and evenness amongst the different allergic conditions. Alpha diversity was measured using Observed, Chao1, Shannon and Simpson indexes (Figs. 4A–C). Observed index reflecting the community richness by counting the unique OTUs showed a significant decrease in the alpha diversity of IgA coated bacteria; between controls and ENP patients (Fig. 4A). No significant difference was observed between controls and ALL or AD patients. Similarly, Chao1 index that estimates the total number of taxa while reflecting the rare OTUs showed a significant decrease of diversity in the ENP patients compared to controls. No significant difference was reflected in the Shannon or Simpson indices (Fig. 4A). In contrast, IgE-coated bacteria showed no significant differences in the alpha diversity in any of the allergic groups compared to controls (Fig. 4B). Among IgG-coated bacteria, the AD group showed decreased alpha diversity using the Shannon and Simpson indices (Fig. 4C).

Next, we examined the beta diversity using the Bray–Curtis distance matrix which revealed that the AD group was significantly distant from the CON and ALL groups in IgA (Fig. 4D), IgE (Fig. 4E), and IgG-coated (Fig. 4F) bacterial groups. The ENP group was significantly distant from the CON group in IgA and IgE bacterial coating (Fig. 4D and E). The IgA coated ENP group also showed significant dissimilarity from the ALL group (Fig. 4D). The AD group showed significant dissimilarity of IgG coating, distinct from CON, ALL and ENP groups (Fig. 4F). Taken together, the above results identified that AD and ENP groups showed a loss of alpha diversity in IgA and IgG subsets and also displayed significant dissimilarity of beta diversity in IgA subset (AD and ENP) and IgG subset (AD) compared to controls.

(See figure on next page.)

Fig. 3 Immunoglobulin coating reveals alterations in levels of coated fecal bacterial OTUs. **A–D** Upset plots showing bacterial taxa across differentially coated subsets within **A** CON **B** ALL, **C** AD and **D** ENP groups. **E** Comparative analysis of total detected bacterial OTUs in the study groups. **F** Ig repertoire diversity index analysis as a measure of the percentage of mono-Ig bound bacteria together with that of the total OTU counts individually for IgA, IgE and IgG coated bacteria [Ig repertoire diversity index = (% of Mono-Ig coating) × (OTU abundance)]. Quantitative variables were compared using Kruskal–Wallis and Dunn's post-hoc analysis. Statistical significance is defined as * $p < 0.05$ and ** $p < 0.01$. Data are represented as mean ± SEM

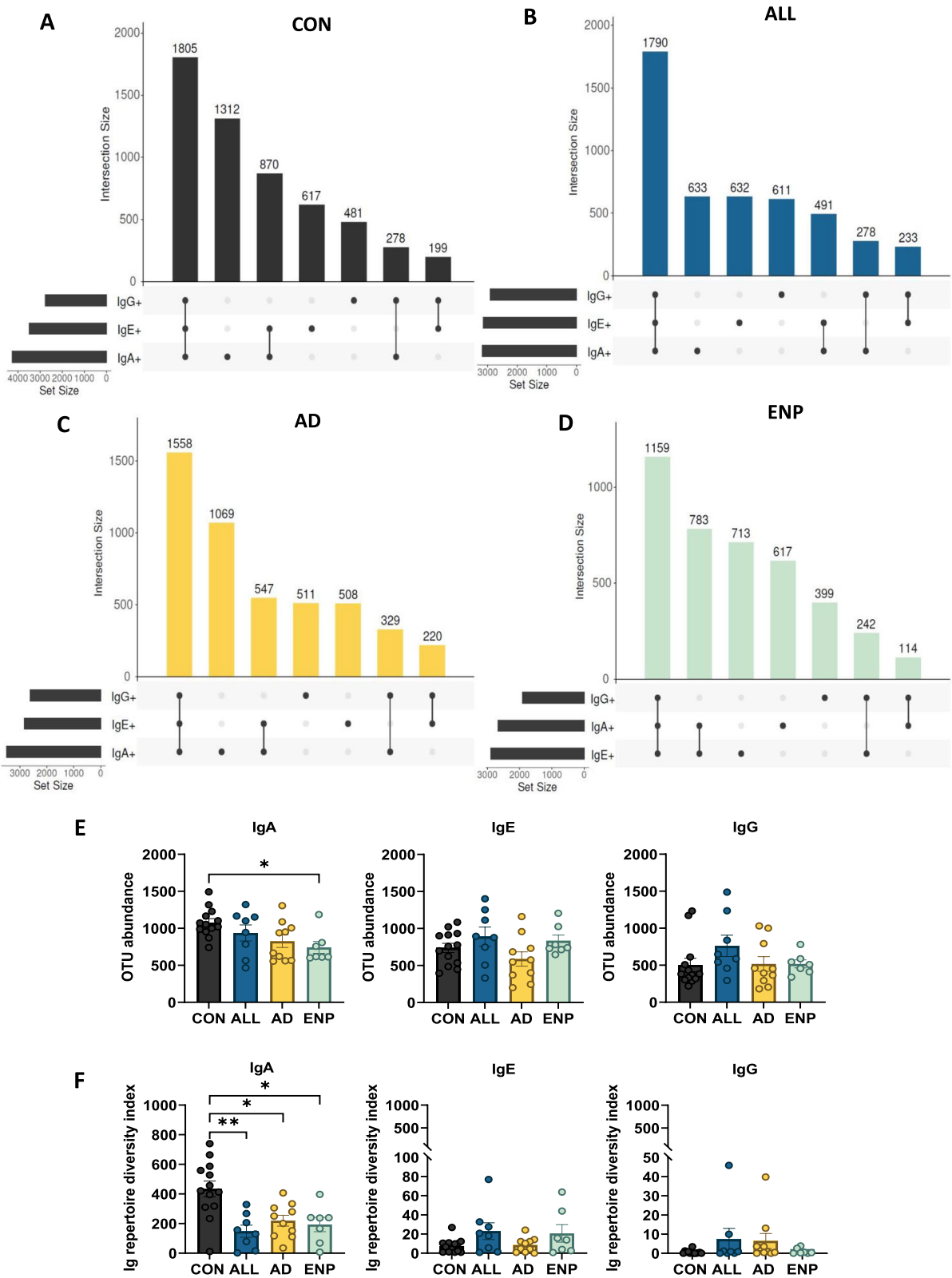


Fig. 3 (See legend on previous page.)

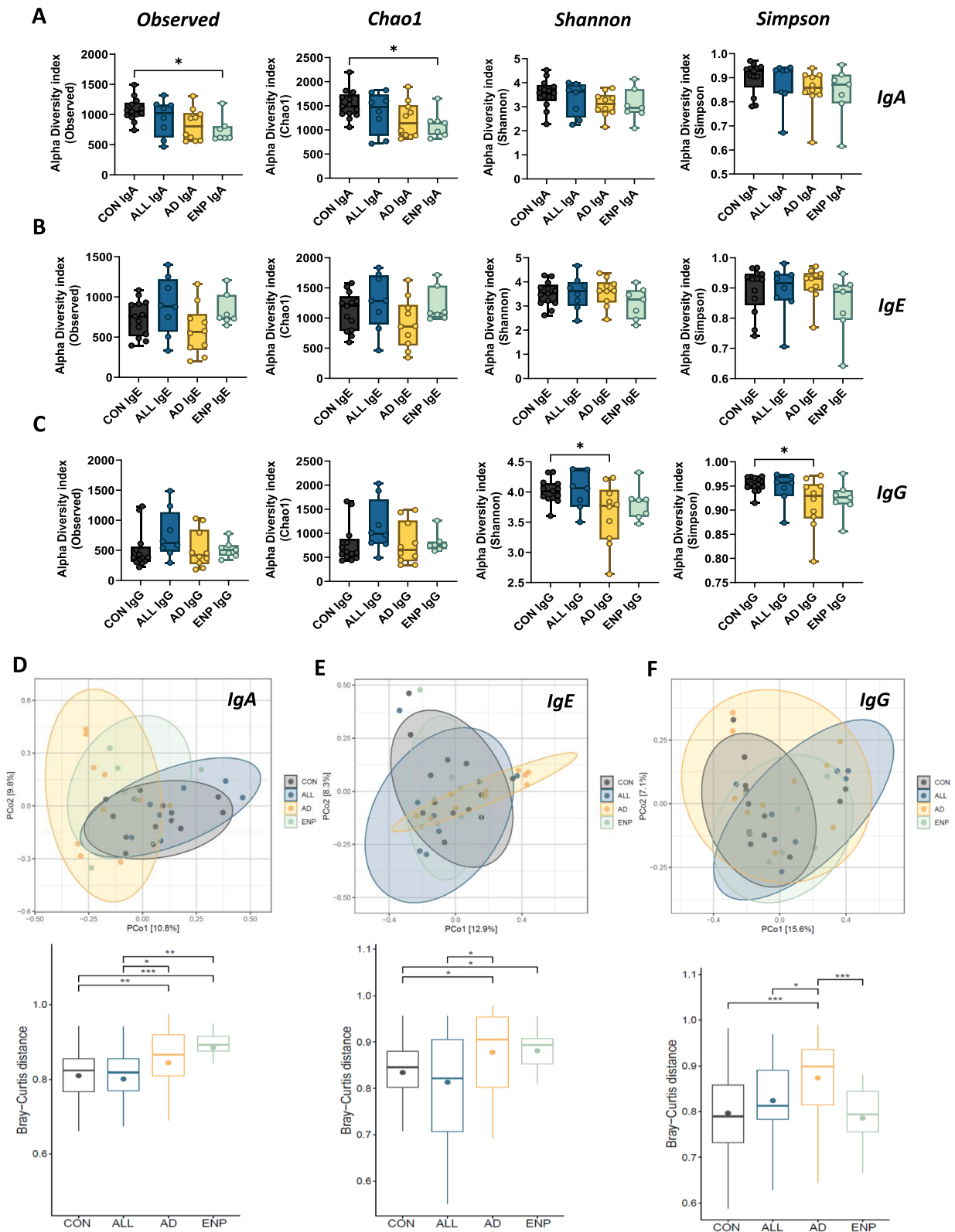


Fig. 4 Alpha and beta diversity in cow milk allergic pediatric subjects. **A** Comparison of alpha diversity in IgA, IgE and IgG groups using Observed, Chao1, Shannon and Simpson indices. **B, C, D** Principal co-ordinate analysis plots of IgA (**B**), IgE (**C**) and IgG (**D**) coated bacteria (above) and box plots for the same (below). Quantitative variables were compared using one-way ANOVA and Dunnet post-hoc analysis for alpha diversity and Wilcoxon test for beta diversity. Statistical significance is defined as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Data are represented as mean \pm SEM

Dysbiosis is associated with differentially coated bacterial subsets in cow milk allergy

We next assessed the bacterial communities coated by IgA, IgE or IgG and identified that the majority of the coated bacteria belonged to the *Firmicutes* and *Bacteroidetes* phyla followed by *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Fusobacteria* in the order of abundance (Fig. 5A). Analysis of the differences in the phyla abundance showed that in the IgA-coated AD group, *Firmicutes* were highly abundant (Fig. 5B and Supplementary Figure S2A), whereas *Verrucomicrobia* was present at a lower abundance as compared to the CON group (Fig. 5B and Supplementary Figure S2D). In the IgE coated subset, *Fusobacteria* was higher in AD group, compared to controls (Fig. 5B and Supplementary Figure S2F). While, in the IgG subset, ALL subjects had a significantly lower abundance of *Bacteroidetes* (Fig. 5B and Supplementary Figure S2B) and *Actinobacteria* in comparison to controls (Fig. 5B and Supplementary Figure S2E).

To assess the dysbiosis associated with the bacterial coating across the different allergic groups, we calculated the ratios of *Firmicutes* in comparison to the other major phyla identified across the Ig-coated subsets. In the IgA-coated bacterial subset, we identified an increase in the ratio of *Firmicutes* to *Bacteroidetes* in the AD group as compared to the CON group (Fig. 5C). The ratio of *Firmicutes* to *Verrucomicrobia*, increased in the AD group compared to controls in the IgA-coated bacterial subset (Fig. 5C). IgG-bound bacteria showed an increase in the ratio of *Firmicutes* to *Bacteroidetes* and an increase in *Firmicutes* to *Actinobacteria* in the ALL group (Fig. 5D). These results align with a previous study which reported an enrichment of *Firmicutes* and decrease of *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* in subjects with food allergies [49].

The levels of dysbiosis associated with bacterial subsets at the OTU level were then evaluated using median CLV score, as described previously [47]. Based on this scoring analysis, we identified bacterial dysbiosis to be associated with IgA, IgE and IgG-bound bacteria in AD subjects, as compared to controls (Fig. 5E). IgA-coated

bacteria in ENP subjects also displayed a significant level of dysbiosis compared to controls (Fig. 5E).

Preferential coating of bacteria of Ig's differs by cow milk allergic phenotype

Next, we explored the bacterial composition at the genus level, to identify their differential abundance among CMA patient subsets. The top 25 abundant bacterial genera across all three subsets—IgA, IgE, and IgG were primarily identified (Fig. 6A). For each individual subset of IgA, IgE and IgG-coated bacteria, the top-most abundant 25 genera are shown in Supplementary Figure S3 A-C. Although *Bacteroides* was identified as the dominant genus within the three Ig subsets (Fig. 6A and Supplementary Figure S3 A-C), we did not identify any significant differences in the binding of *Bacteroides* to IgA, IgE or IgG between the study groups (Supplementary Figure S4). Interestingly, the second most abundant genus, *Faecalibacterium* (Fig. 6A and Supplementary Figure S3 A-C) was found predominantly coated by IgA, IgE and IgG in the ALL group (Fig. 6A and B). *Faecalibacterium* displayed the highest significant differences within the IgE bound subset, with significantly higher coating in the ALL group compared to CON, AD and ENP groups (Fig. 6B). In addition, within IgA and IgG-coated subsets, *Faecalibacterium* showed significantly higher binding in the ALL group compared to AD group (Fig. 6B).

Ruminococcus genus from the family *Lachnospiraceae* was significantly enriched within the IgA coated bacteria from the AD group, as compared to IgA-coated *Ruminococcus* in CON and ALL groups (Fig. 6C). In addition, the abundance of IgA-coated *Ruminococcus* was also significantly greater than IgE and IgG coated *Ruminococcus* (Fig. 6C). *Flavobacterium* showed significantly higher rates of IgE and IgG coating than IgA coating in the AD group (Fig. 6D). Control subjects also exhibited a higher level of IgG-coated *Flavobacterium*, as compared to IgA and IgE coated bacteria (Fig. 6C). Additionally, a second *Ruminococcus* genus from the *Ruminococcaceae* family exhibited significant decreases in IgE coating in the AD group in comparison to the control group (Fig. 6E), and *Staphylococcus* and *Acinetobacter* were found to be predominantly IgG-coated in the AD group (Fig. 6F and

(See figure on next page.)

Fig. 5 Dysbiosis is associated with differential bacterial Ig coating in CMA subjects. **A** Most abundant phyla among the mono- IgA, IgE and IgG coated bacteria. **B** Heat-map showing the significant differential distribution of phyla recognition patterns in IgA, IgE and IgG subsets. Each subset has been compared with the control group in the respective subsets. **C, D** Quantitative analysis of dysbiosis at phyla level analyzing *Firmicutes*/*Bacteroidetes* and *Firmicutes*/*Verrucomicrobia* in IgA coated (**C**) and *Firmicutes*/*Bacteroidetes* and *Firmicutes*/*Actinobacteria* in IgG coated bacteria (**D**). **E** Quantitative analysis of dysbiosis using Median CLV dysbiosis score in IgA, IgE and IgG coated bacteria. Quantitative variables were compared using Kruskal–Wallis and Dunn's post-hoc analysis for panels **B–D** and one-way ANOVA and Dunnett post-hoc analysis for panel **E**. Statistical significance is defined as * $p < 0.05$, ** $p < 0.01$. See also Additional file 1, Supplementary Figure S2. Data are represented as mean \pm SEM

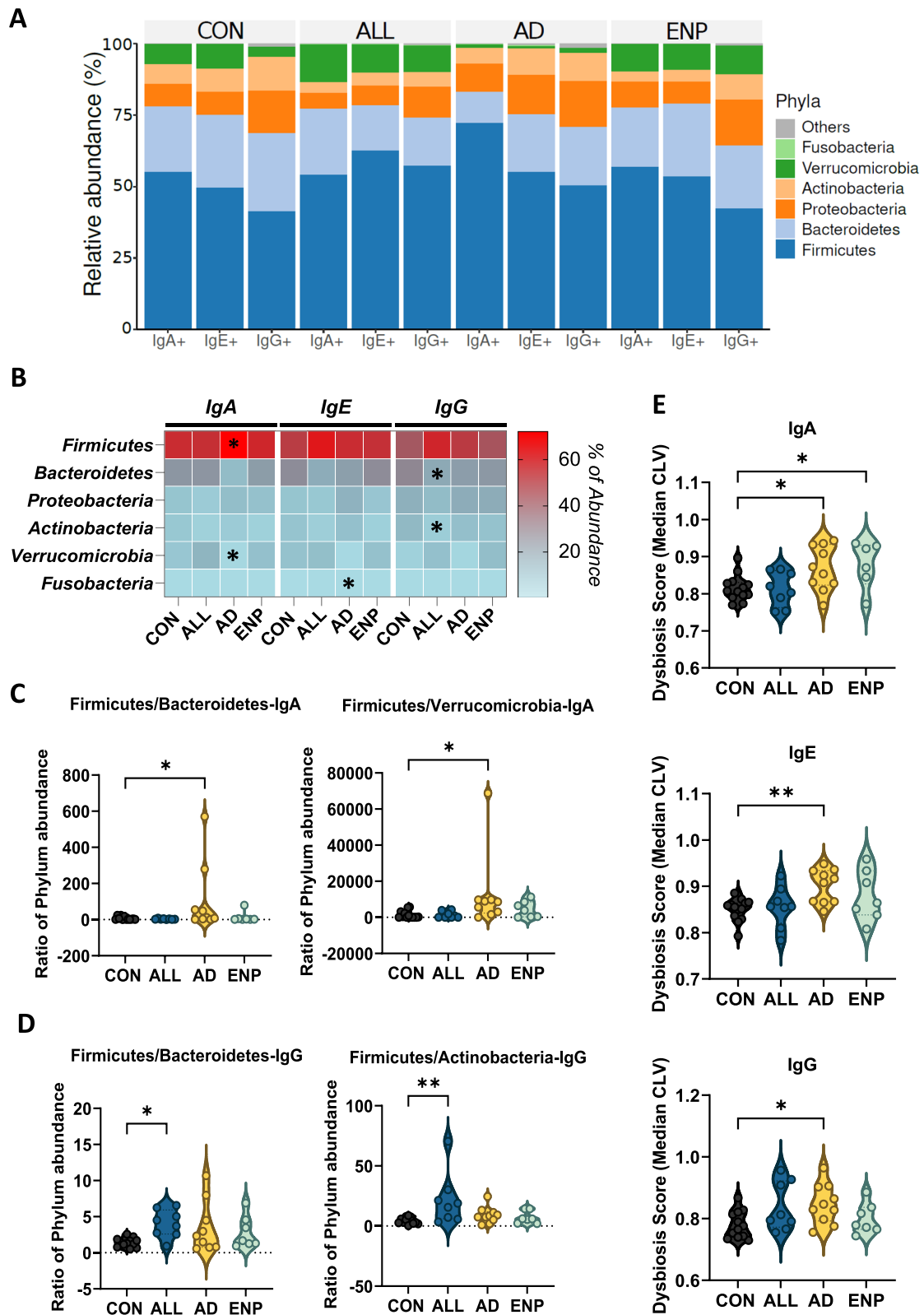


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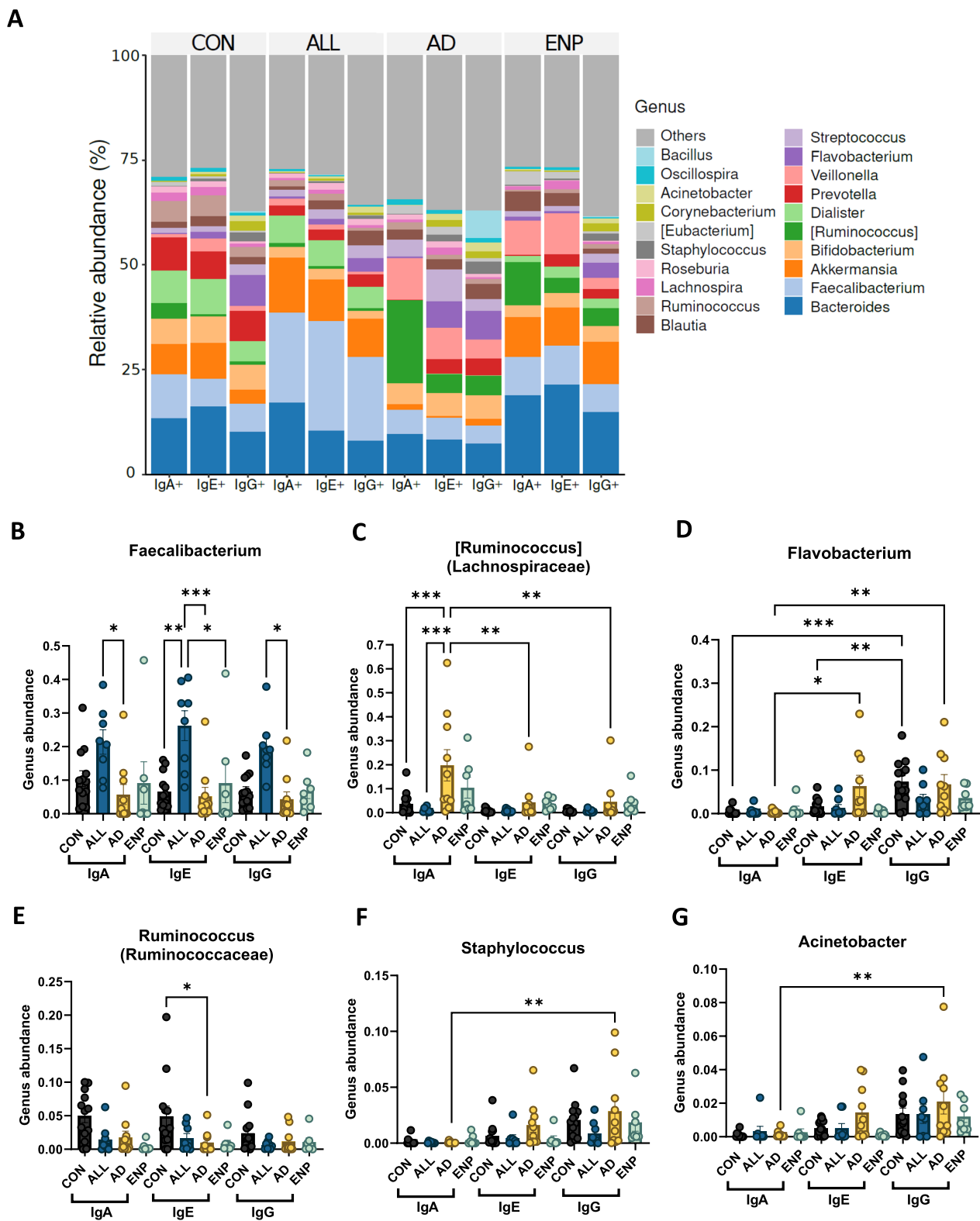


Fig. 6 Preferential bacterial coating of most abundant bacteria in CMA subjects. **A** Top 25 most abundant bacterial genera across mono-IgA, IgE and IgG coated bacteria. **B–G** Quantitative analysis of differential abundance of IgA, IgE and IgG coated **B** *Faecalibacterium*, **C** *Ruminococcus* from *Lachnospiraceae* **D** *Flavobacterium* **E** *Ruminococcus* from *Ruminococcaceae* **F** *Staphylococcus* and **G** *Acinetobacter*. Quantitative variables were compared using two-way ANOVA and Tukey post-hoc analysis. Statistical significance is defined as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Data are represented as mean \pm SEM. (See also Supplementary Figure S3 and Supplementary Figure S4)

G). *Akkermansia*, *Bifidobacterium*, *Veillonella*, *Dialister*, *Prevotella*, *Streptococcus*, *Blautia*, *Lachnospira*, *Roseburia* and *Eubacterium* did not show significant changes in their preferences of binding to IgA, IgE or IgG (Supplementary Figure S4). However, *Corynebacterium* showed significant difference between IgA and IgG bound bacteria in the control group (Supplementary Figure S4).

Identification of uniquely dominant bacterial targets of Ig response in CMA groups

To further identify the bacterial targets of Ig's at the species level, we identified the unique and common bacterial species bound by IgA, IgE and IgG subsets present in the study groups (Fig. 7A). Subsequently we characterized the most abundant bacterial targets bound by specific Ig's across the study groups, LEfSe analysis was conducted at the species level, allowing the determination of the bacterial species that were most predominantly coated by an individual Ig in each of the different study groups (Fig. 7B–D). The most abundant uniquely IgA coated bacterial subsets within the CON group were *Dialister*, *Ruminococcus*, *Bacteroides uniformis*, *Roseburia*, *Ruminococcus bromii*, *Dorea formicigenerans*, *Roseburia faecis* and *Streptococcus anginosus* (Fig. 7B). *Roseburia* species, *Ruminococcus bromii* have been previously identified to be highly abundant in non-allergic conditions [50]. Interestingly, in the CON group, we identified predominance of only two bacterial species in the IgE coated subset, namely, *Dialister* and an unclassified genus from the *Aerococcaceae* family, and none in the IgG-coated subset (Fig. 7C and D).

Faecalibacterium prausnitzii was identified as coated by IgA, IgE and IgG in the ALL group (Fig. 7B–D). We also determined that *Eubacterium dolichum* was predominantly coated by IgA, IgE and IgG in the ENP group (Fig. 7B–D). *Ruminococcus gnavus* was found to be predominantly coated with IgA in the AD group and with IgE in the ENP group (Fig. 7B and C). *Bacteroides fragilis*, a species from *Barnesiellaceae* family, *Slackia*, *Collinsella aerofaciens*, a second *Collinsella* species, a species from phyla *Alphaproteobacteria* and *Lachnospira* were all found to be most abundant bacteria among the IgA coated bacteria in the ALL group (Fig. 7B). In addition to *Ruminococcus gnavus*, the IgA coated AD group showed predominance of a species from *Erysipelotrichaceae*, *Blautia producta*, *Eggerthella lenta*, *Prevotella melanogenica*, *Lactobacillus zae*, an unclassified *Eggerthella* species, two unclassified species from order *Lactobacillales* and family *Alcaligenaceae*, *Proteus* and *Pseudoxanthomonas taiwanensis* (Fig. 7B). The AD group displayed the highest number of IgA coated species identified, as compared to the rest of the subject groups (Fig. 7B). In addition to the presence of *Eubacterium dolichum*, in the

IgA coated ENP group, we identified the presence of *Lautropia*, *Clostridium hathawayei*, *Robinsonella peoriensis*, two unclassified species from order *CW040* and a member of the *Legionellaceae* family (Fig. 7B).

Among the IgE-coated bacteria, we identified the highest number of uniquely coated bacterial OTUs (Fig. 3D) and highest number of uniquely bound bacterial species (Fig. 7A) present in the ENP group. While *Roseburia*, *Ruminococcus bromi*, *Coprococcus eutactus*, *Slackia*, a species from *Halomonadaceae* family and *Lachnobacterium* were found to be highly IgE coated in the ALL group, *Fusobacterium*, *Curvibacter* and *Helicobacter* were most abundant in the IgE coated AD group samples (Fig. 7C). In the ENP group, following *Ruminococcus gnavus*, *Eubacterium dolichum* was found to be predominantly IgE-coated followed by *Veillonella*, *Clostridium neonatale*, *Clostridium citroniae*, *Robinsoniella peoriensis*, two unclassified species from *Rhizobiaceae* and *Ellin6075*, *Agrobacterium* and *Citrobacter* (Fig. 7C).

Bacterial IgG coating in the ALL group was found to be highest for *Dialister* and *Blautia producta*, both of which followed *Faecalibacterium prausnitzii* (Fig. 7D). *Corynebacterium lubricantis* and *Eggerthella* were predominantly IgG coated in the AD group (Fig. 7D). The elevated proportion of IgG bound bacteria observed in the AD group could potentially have been driven by the presence of these pathogenic bacteria. *Hydrogenophilus* and *Brevundimonas diminuta*, in addition to *Eubacterium dolichum* were found to be highly IgG coated in the ENP group (Fig. 7D).

To understand the specificity of the biomarkers identified in each of the allergic groups and Ig subsets, the area under the ROC curve (AUC) analysis for IgA, IgE and IgG subsets was performed, using the bacterial biomarkers identified from the LEfSe analysis in Fig. 7B–D. Analysis of the AUC values for the IgA-coated subset generated values of 0.61, 0.72, 0.92 and 0.89 for CON, ALL, AD and ENP groups respectively (Fig. 7E). For the IgE-coated CON, ALL, AD and ENP groups, AUC values were 0.86, 0.78, 0.75 and 0.67 respectively (Fig. 7F). The IgG-coated subset gave AUC values of 0.71, 0.89, 0.79 and 0.94 respectively for the CON, ALL, AD and ENP groups (Fig. 7G). As such, these results indicate that the Ig-specific binding of the identified microbes (Fig. 7B–D) are reliable biomarkers associated with IgE and non-IgE mediated cow milk allergy.

Discussion

In this study, we sought to identify the presence of differential Ig-specific bacterial coating patterns of IgA, IgE and IgG to gut bacteria in pediatric CMA subjects and to discern whether there are disease specific patterns of humoral responses through the application of

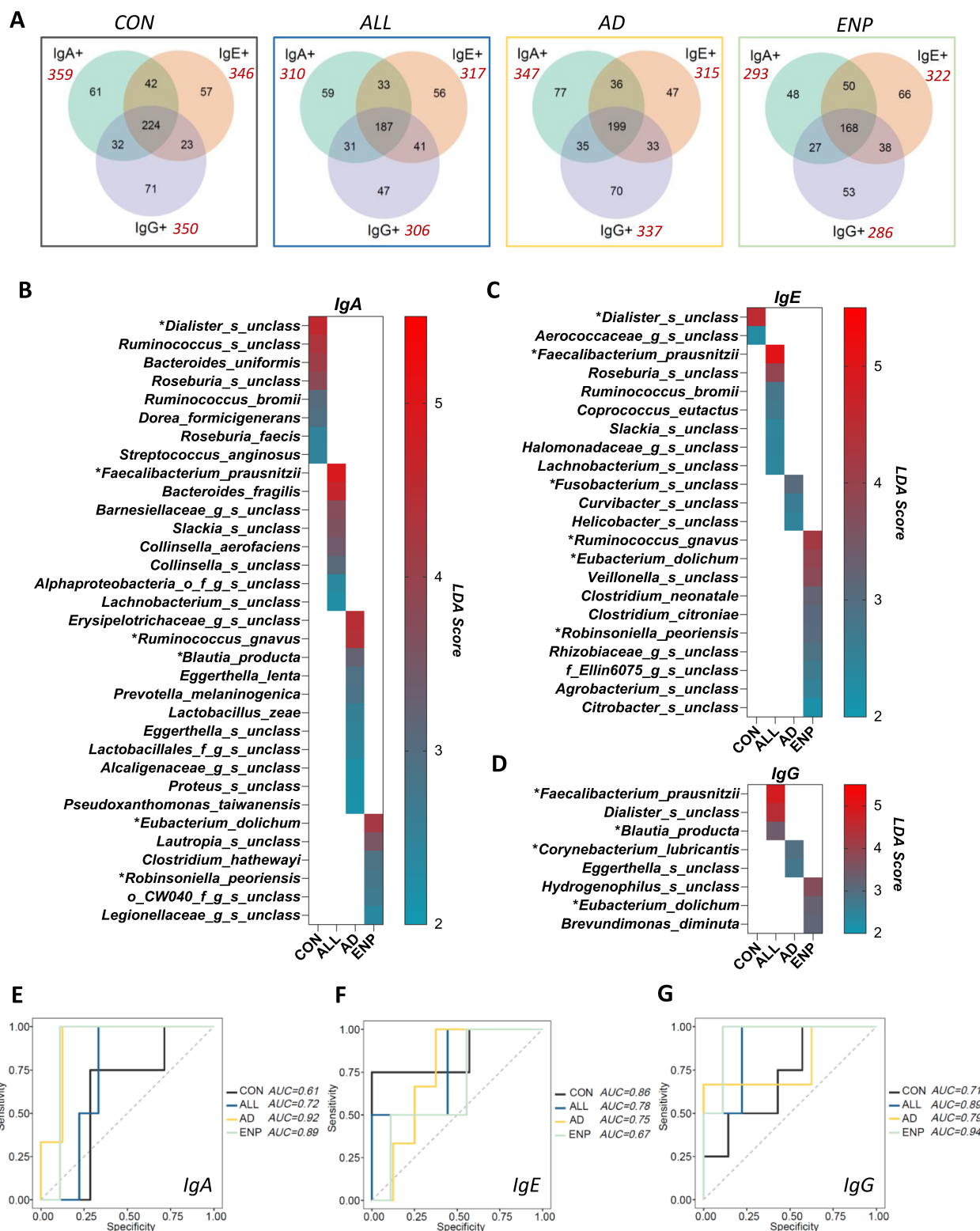


Fig. 7 Identification of uniquely dominant bacterial targets of Ig response in CMA groups. **A** Unique and common bacterial species present in IgA, IgE and IgG subsets among the study groups. Total number of bacterial species bound by each Ig subset in the different study groups are shown in red. **B–D** Lefse analysis showing uniquely abundant bacteria with LDA score above 2 within **B** IgA, **C** IgE and **D** IgG coated bacteria. **E–G** ROC curve analysis showing the AUC analysis for **E** IgA, **F** IgE and **G** IgG coated bacteria

Ig based bacterial sorting and subsequent 16S rRNA gene sequencing. A thorough understanding of the patterns of antibody responses generated against specific gut bacteria may aid in understanding the functional relevance of Ig's in allergic disease and could potentially be developed as biomarkers for the severity of allergy or as a therapeutic target for modulating responses in specific disease settings. It has been previously shown that elevated intestinal IgA levels are protective against allergic diseases [37]. Moreover, IgA has been identified by several groups as an important factor for the maintenance of intestinal homeostasis, by inhibiting bacterial virulence and through shaping gut microbial composition to promote colonization by symbiotic bacteria [51–53]. The microbiota in the gut can also steer IgA production [54] which controls bacterial translocation and enables neutralization of bacteria toxins at the mucosal surface [55]. Our preliminary results identified a significantly decreased proportion of unique and total IgA bound bacteria in both IgE and non-IgE mediated CMA conditions by pairwise comparison in control and allergic subjects. Furthermore, quantification of the unique and total IgA coating in allergic subjects in comparison to non-allergic control subjects identified a significant decrease in bacterial IgA coating for IgE mediated ALL and non-IgE mediated ENP groups. An elevated level of mono-IgG and total IgG bacterial coating was identified as a unique feature for the IgE mediated AD group. AD subjects also exhibited a significant increase in IgA + IgG + and IgE + IgG + dual coated bacteria. Notably, the IgE coating of bacteria in the gut, did not show any differences between CON and the IgE mediated allergic groups, despite the presence of significantly greater levels of IgE in plasma for the ALL and AD groups (Table 2). Although the presence of IgE in the gut has previously been reported to be positively correlated between plasma and fecal samples in patients with atopic allergic disease [56] and those with parasitic infections [57, 58], our results indicate that the levels of plasma IgE present, may not directly correspond with the degree of bacterial IgE coating in the gut. Moreover, these studies have either focused on total levels of free IgE [57] or on the presence of allergen specific IgE and not specifically on gut bacteria coated by IgE [59]. Additionally, it has been shown that IgE could act as an antigen/allergen transepithelial transporter in combination with CD23 indicating it may play a distinct role in gut associated humoral responses [60]. To our knowledge the only prior study to directly assess IgE binding of bacteria in the human gut was a recent report by Abdel-Gadir et al., where, in contrast to our findings, they identified increased levels of bacterial IgE coating in IgE mediated food allergic subjects [38]. The disparity in these findings may potentially be due to

differences in study populations including their younger age of recruitment, higher levels of multi-sensitization, or other clinical factors. Additionally, it should be noted that in the murine model employed by Abdel-Gadir et al. they observed that allergic sensitization with OVA and staphylococcal enterotoxin B, did not lead to an increase in the levels of IgE coated bacteria present in wild type animals. An additional study by Parrish et al. [39] also assessed IgE binding to gut microbes in a murine model. Here, increased levels of IgE coated bacteria were found to be present after five weeks on a fiber free diet, however this difference was only detectable in fecal content and was not detectable in ileal content. Taken together, these data provide an initial insight into how altered humoral immune responses to gut bacteria induced by the onset of food allergic conditions may be important in shaping the course of disease. However, many questions still remain unanswered as to the exact role of IgE mediated immune responses in the gut, especially in relation to potential microbiome specific responses, how IgE-producing memory B cells or plasma cells are generated, and whether IgE production is localized to the gut [61, 62] or is transported from the plasma [63].

The overall decrease in alpha diversity and dysbiosis we identified in association with the CMA subjects could potentially be due to the altered levels of IgA and IgG detected in these subjects. Alternatively, the increased prevalence of pathogenic bacteria identified under allergic conditions including, *Bacteroides fragilis*, *Collinsella aerofaciens* (IgA-ALL), *Ruminococcus gnavus* (IgA-AD), *Clostridium hathewayi*, *Robinsonella peoriensis* (IgA-ENP), could have contributed to decreases in IgA levels in these CMA subjects, through previously identified mechanisms such as the proteolytic cleavage of IgA which results in a decreased proportion of IgA bound bacteria [64, 65]. We also observed an increase in IgG bound bacteria in the AD group, possibly due to the increased abundance of pathogenic bacteria identified in this group, namely, *Staphylococcus*, *Acinetobacter* and *Corynebacterium*.

Following analysis of the 20 most abundant genera bound by IgA, IgE or IgG, we identified significant differences in the bacterial coating of *Faecalibacterium prausnitzii*, *Ruminococcus gnavus*, *Flavobacterium*, *Ruminococcus bromii*, *Staphylococcus* and *Acinetobacter* among the CMA groups. Our study found *Staphylococcus* and *Corynebacterium* to be more highly recognized by IgG, which is consistent with the established roles of *Staphylococcus* [66] and *Corynebacterium* [67] in atopic dermatitis. Moreover, atopic dermatitis has been reported to precede the development of food allergy [68] and skin colonization with *Staphylococcus aureus* has been shown to increase the susceptibility to food allergy

[69]. Our study indicates that *Staphylococcus aureus* is also more prevalent in the gut of IgE mediated CMA subjects presenting with atopic dermatitis, which was observed to be predominantly bound by IgG suggesting the induction of an inflammatory response. We also identified an increased abundance of IgG coated *Acinetobacter* in the AD group (Fig. 6G). *Acinetobacter* was in one instance shown to be associated with protection against allergic sensitization and inflammation [70]. However, it has also been alternately reported that *Acinetobacter baumannii*, *Acinetobacter lwoffii* and *Acinetobacter haemolyticus* are associated with an increased incidence of AD [71].

Faecalibacterium prausnitzii has been previously found to be abundant in food and respiratory allergic conditions [50] and has also been shown to be strongly associated with atopic dermatitis [72]. Our observation strengthens these previous studies which have identified an increased abundance of *Faecalibacterium prausnitzii* in allergic conditions. Likewise, our results show *Eubacterium dolichum* to be highly bound by IgA, IgE and IgG in the ENP group, aligning with a previous study on *Eubacterium dolichum* which reported an increased bacterial burden in a mouse model of food allergy [73]. *Eubacterium dolichum*, hence could be a potential biomarker for non-IgE mediated cow milk allergy. *Robinsoniella peoriensis*, identified as highly IgA and IgE coated in the ENP group, could also be a potential biomarker for determining a predisposition to non-IgE mediated cow milk allergy. Further, *Ruminococcus gnavus* has been previously shown to be associated with increased allergic conditions in infants [74] and children [50] and a pathogenic role for *Ruminococcus gnavus* has also been found in association with diarrhea-predominant irritable bowel syndrome, through increasing serotonin biosynthesis [75]. *Bacteroides fragilis* was identified as highly coated by IgA in the ALL group (Fig. 7A) and although a previous study has reported the role of *Bacteroides fragilis* in the maintenance of tolerance [76], its increased abundance has also been associated with peanut and nut-allergic patients [77].

Conclusions

Although IgE mediated allergic diseases have been previously studied in association with the microbiome, non-IgE mediated food allergies are not yet well described, and their microbial association has not been clearly established. Our results have identified significant differences in how the bacterial Ig coating with respect to IgA, IgE and IgG varies in pediatric IgE and non-IgE mediated cow milk allergic conditions. This

is the first-time bacterial Ig coating has been studied in the context of a non-IgE mediated allergy. Here, we report a previously unidentified association between decreased IgA coating and non-IgE mediated CMA. We also identify an elevated IgG binding in subjects presenting with atopic dermatitis associated with IgE mediated CMA. Moreover, we show the existence of specific gut bacterial signatures that are preferentially bound by IgA, IgE and IgG in both IgE and non-IgE mediated CMA conditions. Hence, the results from our study, provide further evidence that alterations in humoral responses that result in dysbiosis may be an underlying cause of allergic disease; raising the prospect for developing new insights into the clinical diagnosis of non-IgE mediated allergic conditions, and the design of future therapeutic interventions, by targeting the bacterial species identified here.

Abbreviations

CMA	Cow milk allergy
Ig	Immunoglobulin
CON	Healthy controls
ALL	IgE mediated CMA subjects without atopic dermatitis
AD	IgE mediated CMA subjects with atopic dermatitis
ENP	Non-IgE mediated CMA subjects
FPE	Food protein-induced allergic enteropathy
FPIES	Food protein-induced enterocolitis syndrome
FPIAP	Food protein-induced allergic proctocolitis
IBD	Inflammatory bowel disease
OTU	Operational taxonomic unit
LEFSe	Linear discriminant analysis effect size

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-024-05850-z>.

Additional file 1

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Author contributions

All listed authors contributed to this study. TA, FB and NVP contributed to the study design. TA, FB and SM contributed to study execution. AA, ME and MA enrolled patients and recorded clinical information. FB performed the consenting, collection of questionnaires and coordinated sample collection. TA performed flow cytometry assays, bacterial sorting and analysis, and DNA isolation from sorted Ig coated bacterial cells. JC and GG contributed to design and optimal sorting of Ig coated bacterial cells. SM performed 16S rRNA gene sequencing and analysis. TA and NVP wrote the manuscript. TA, SM and NVP contributed to data analysis. TA, SM, JC, SAK and NVP engaged in study interpretation and critical analysis. All authors reviewed the manuscript.

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Availability of data and materials

16S rRNA gene sequencing analysis data are deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1127830 [(Run Selector: NCBI (nih.gov), SRP520730-SRA-NCBI (nih.gov)].

Declarations

Ethics approval and consent to participate

The study obtained written parental consent from all participants before enrollment. The study protocol (1500751) was approved by the Institutional Review Board (IRB) at Sidra Medicine, Doha, Qatar

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial or non-financial interests.

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