Author(s): Mella, Miia A.; Lavrinienko, Anton; Akhi, Ramin; Hindström, Rasmus; Nissinen, Antti E.; Wang, Chunguang; Kullaa, Arja; Salo, Tuula; Auvinen, Juha; Koskimäki, Janne J.; Hörkkö, Sohvi

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RESEARCH ARTICLE

Innate Immunity

Compensatory IgM to the Rescue: Patients with Selective IgA Deficiency Have Increased Natural IgM Antibodies to MAA–LDL and No Changes in Oral Microbiota

Miia A. Mella,* Anton Lavrinienko,[†,‡] Ramin Akhi,* Rasmus Hindström,[‡] Antti E. Nissinen,* Chunguang Wang,[§] Arja Kullaa,[¶] Tuula Salo,[‖,#,,††] Juha Auvinen,[‡‡,§§] Janne J. Koskimäki,[‡,1] and Sohvi Hörkkö*[,1]**

*Medical Microbiology and Immunology, Research Unit of Biomedicine, University of Oulu, Oulu, Finland; [†]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland; [‡]Ecology and Genetics Research Unit, University of Oulu, Oulu, Finland; [§]Cardiovascular Research Unit, Minerva Foundation Institute for Medical Research, Helsinki, Finland; [¶]Department of Dentistry, School of Medicine, University of Eastern Finland, Kuopio, Finland; [‖]Translational Immunology Research Program, University of Helsinki, Helsinki, Finland; [#]Department of Oral and Maxillofacial Diseases, Clinicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland; **Cancer and Translational Medicine Research Unit, Faculty of Medicine, University of Oulu, Oulu, Finland; [††]Department of Pathology, University of Helsinki, Helsinki, Finland; [‡‡]Center for Life Course Health Research, University of Oulu, Oulu, Finland; and [§§]Medical Research Center, Oulu University Hospital, Oulu, Finland

ABSTRACT

IgA is the most abundant Ab in the human body. However, most patients with selective IgA deficiency (SIgAD) are asymptomatic. IgM, and to lesser extent IgG Abs, are generally presumed to compensate for the lack of IgA in SIgAD by multiplying and adopting functions of IgA. We used data from the Northern Finland Birth Cohort 1966 to investigate whether SIgAD patients have differences in levels of natural Abs to oxidized epitopes compared with 20 randomly selected healthy controls. First, we screened the saliva and serum samples from the Northern Finland Birth Cohort 1966 cohort ($n = 1610$) for IgA concentration. We detected five IgA-deficient subjects, yielding a prevalence of 0.3%, which is consistent with the general prevalence of 0.25% in the Finnish population. To detect natural Abs, we used malondialdehyde acetaldehyde–low-density lipoprotein (MAA–LDL), an Ag known to bind natural Abs. In this study, we show that natural secretory IgM and IgG Abs to MAA–DL were significantly increased in subjects with SIgAD. Given that secretory IgA is an important part of mucosal immune defense and that, in the gut microbiota, dysbiosis with SIgAD patients has been observed, we characterized the oral bacterial microbiota of the subjects with and without SIgAD using high-throughput 16S rRNA gene sequencing. We found no significant alterations in diversity and composition of the oral microbiota in subjects with SIgAD. Our data suggest that increased levels of secretory natural Abs in patients with SIgAD could be a compensatory mechanism, providing alternative first-line defense against infections and adjusting mucosal milieu to maintain a healthy oral microbiota. *ImmunoHorizons*, 2021, 5: 170–181.

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Address correspondence and reprint requests to: Miia Mella, Medical Microbiology and Immunology, Research Unit of Biomedicine, University of Oulu, Aapistie 5 A, 90220 Oulu, Finland. E-mail address: miia.mella@oulu.fi

ORCIDs: 0000-0003-1783-5330 (R.A.); 0000-0003-4771-2753 (A.E.N.); 0000-0001-5569-321X (C.W.); 0000-0001-8198-8185 (A.K.); 0000-0001-6039-0088 (T.S.); 0000-0001-8241-3500 (J.J.K.); 0000-0003-2118-5270 (S.H.).

[1]J.J.K. and S.H share senior authorship.

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Abbreviations used in this article: ASV, amplicon sequence variant; BMI, body mass index; CVID, common variable immunodeficiency; FIMM, Institute for Molecular Medicine Finland; LDL, low-density lipoprotein; MAA, malondialdehyde acetaldehyde; MDA, malondialdehyde; NFBC1966, Northern Finland Birth Cohort 1966; OGTT, oral glucose tolerance test; PERMANOVA, permutational ANOVA; RLU, relative light unit; SIgAD, selective IgA deficiency

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INTRODUCTION

Natural Abs are defined as Abs that are generated in the absence of exogenous antigenic stimulation (1, 2). They consist mainly of IgM but can also be of IgA and IgG subclasses (2, 3). Natural Abs form the first-line humoral defense against pathogens and possess immunoregulatory functions. They are nonspecific and polyreactive in the sense that they cross-react with identical epitopes shared between pathogens and self-antigens (1, 3). An example of such an epitope is phosphorylcholine, which can be found both within the cell wall of *Streptococcus pneumoniae* and in apoptotic cells or oxidized lipids (1, 3). Thus, in addition to protection against pathogens, natural Abs provide homeostatic housekeeping functions and participate in removal of altered self-antigens that are formed during apoptosis or oxidation (2, 4).

Another epitope found in oxidized lipids and recognized by natural Abs is malondialdehyde acetaldehyde (MAA) adduct (5). MAA adducts are stable and immunogenic reaction products of the highly reactive aldehydes acetaldehyde and lipid peroxidation-derived malondialdehyde (MDA) (6, 7). Under conditions of lipid oxidation, low-density lipoprotein (LDL) can be modified by MDA and MAA adducts, which further creates MDA–LDL and MAA–LDL (8). After MDA modification of LDL in some diseases, such as atherosclerosis, it has been shown that the MAA adduct is the dominant epitope (5). Previously, we have shown that newborns already have natural Abs binding to MAA–LDL, and those Abs are presumed to regulate apoptotic clearance during fetal development and participate in immunomodulation of atherosclerosis (8).

An average human body produces ~60 mg/kg IgA per day, which is more than all other Ig classes combined (9). In selective IgA deficiency (SIgAD), production of IgA is disturbed because of, for instance, maturation defect in B cells (10, 11). SIgAD is defined as a serum IgA level of <7 mg/dL in individuals age 4 y or older with normal serum levels of IgM and IgG and exclusion of other forms of immunodeficiency (12, 13). SIgAD is the most common primary immunodeficiency. In Caucasians, the frequency ranges from 1:142 to 1:965 (12); in Finland, the frequency is ~1:400 (14, 15). Patients with SIgAD are at higher risk to develop autoimmune disorders (10, 12). Also, frequent respiratory infections can occur, especially in SIgAD patients with concomitant IgG subclass deficiency (11, 16). In some cases, SIgAD can also develop into common variable immunodeficiency (CVID) (13). Most patients with SIgAD are, however, asymptomatic. One plausible explanation is that most patients with SIgAD have an increased production of secretory IgM, which has functional similarities with IgA (10, 11). Also, compensatory elevation in IgG levels in SIgAD patients has been observed (17–19). An enhancement in IgG reactivity to phosphorylcholine was reported in a study comparing SIgAD patients and healthy controls (20).

The oral cavity accommodates numerous microorganisms, including bacteria, fungi, viruses, and protozoa (21). It has the second most diverse microbiota after the gut, with over 700 species of bacteria (22, 23). Only a fraction of these bacterial species can be isolated and cultured in a laboratory and have formal nomenclature. However, recent advances in sequencing technologies and rapid development of computational tools provide methods for culture-independent identification and quantification of human-associated microbial communities (24). The oral cavity provides several distinct habitats for microbial colonization, including buccal mucosa, keratinized gingiva, hard palate, tongue, tonsils, throat, sub- and supragingival plaques (22, 25). These complex habitats support the growth of different microbial communities and site-specific specialist species, and these indigenous species prevent pathogen expansion (22, 26). Salivary microbiota is a mixture of microorganisms (mostly bacteria) flushed from all oral sites (27). In saliva and other secretions, IgA is the most abundant Ig (28) and has a prominent role in protecting the host against infections (29). Recent studies have shown that IgA also has a role in shaping the gut microbiota and that SIgAD patients have an altered gut microbiota composition compared with healthy controls (30–32). Also, dysbiosis in the salivary microbiota of IgA-deficient mouse was detected in a recent publication (33). However, no study has quantified the effect of IgA deficiency on the composition of oral microbiota in humans.

In this study, we aim to analyze the natural Ab levels to oxidized epitopes in subjects with IgA deficiency and subjects with normal IgA Ab levels. MAA–LDL and phosphocholine were used as Ags to detect natural Abs. Furthermore, we used amplicon sequencing of bacterial 16S rRNA genes to compare oral microbiota of subjects with IgA deficiency and subjects with normal IgA Ab levels.

MATERIALS AND METHODS

Northern Finland Birth Cohort 1966

The Northern Finland Birth Cohort 1966 (NFBC1966) is a longitudinal and epidemiological research program comprising 12,231 children with expected date of birth in 1966 in the provinces of Oulu and Lapland (34). The objective of the main study was to explore the long-term morbidity, mortality, social welfare, and health behavior of individuals throughout their lives and to discover risk factors and prognostic factors for enabling early intervention and preventive procedures. The data have been collected by questionnaires and clinical examinations and from national registers and health care records. Follow-up studies have been performed at the ages of 1, 14, 31, and 46 y.

The latest follow-up study was carried out in 2012–2014, when the cohort subjects were 45–48 y old. All subjects who were living in Finland and whose address was known (n = 10,321) received postal questionnaires and were invited to a clinical examination. Altogether, 6,868 (66.5%) answered questionnaires, and 5,861 (56.7%) participated in clinical examinations, including for example, anthropometric measurements, cardiovascular, musculoskeletal, and dermatological examinations. Subjects living within 100 km of the city of Oulu (n = 3150)

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were invited into more extensive clinical examinations, which also included dental health examinations and collection of saliva samples. Of this subpopulation, 1,964 (62.3%) subjects participated. Participation was voluntary, and a written consent was obtained from participants. The study has been approved by the ethical committee of North Ostrobothnia District University Hospital and followed the guidelines of the Declaration of Helsinki. Clinical examination included dental health examinations and collection of biological samples such as blood and saliva.

Study subjects

In the current study, we included 1610 cohort subjects from the 46-y follow-up study who went through dental examination and who were able to produce saliva samples. We performed natural Ab level measurements from the serum and stimulated saliva samples of those 1610 study subjects. We quantified the oral microbiota of 972 out of 1610 study subjects who went through dental examination and a glucose tolerance test. Oral microbiota was defined with resting saliva samples.

The IgA levels were first screened in the whole study cohort, and out of the 1610 subjects, five study subjects had a serum IgA level below the definition limit of SIgAD. These subjects were identified as the IgA-deficiency group. The oral microbiota of each of these five subjects had been defined. A control group was formed by randomly selecting 20 control subjects out of the 972 subjects with normal IgA Ab levels and for which the oral microbiota data were available. Each of the 25 study subjects had given a written consent on using their unidentified data in scientific study.

Collection of samples

In the current study, we examined the saliva and serum samples collected in the NFBC1966 46-y follow-up study. Also, data from blood pressure measurements and oral glucose tolerance test (OGTT) were observed for construction of baseline characteristics of the study subjects.

Resting saliva samples were collected over a period of 15 min. First, subjects were instructed to swallow the existing saliva. Then, sitting in a forward leaning position, subjects were told to let saliva quietly drain to a collection container and at 15 min let all existing saliva fall to the container. For stimulated saliva samples, subjects were instructed to chew a block of paraffin for five minutes, during which time the produced saliva was collected. Samples were centrifuged at 1200 rpm for 20 min at 4°C. Supernatant and pellet were separated, and the samples were stored at −80°C for later use.

Blood tests were collected by laboratory personnel after an overnight (12 h) fasting period. Subjects were lying down, and samples were collected from the antecubital vein. Samples were centrifuged at 2200 × g for 11 min. Fresh samples were stored at 4°C before analyzing and samples intended to freeze were frozen at −20°C.

OGTT was also performed after overnight fasting (12 h). Measurement of plasma glucose was done at baseline, 30, 60, and 120 min after the intake of 75 g of glucose. OGTT was not performed for subjects with glucose-lowering medication or fasting capillary glucose over 8 mmol/l. Brachial blood pressure was measured with an automatic oscillometer blood pressure device (Omron Digital Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan) and right-sized cuff on the right arm of seated participants after 15 min of rest. Both systolic and diastolic blood pressure were measured three times with 1 min intervals. The values of the third measurement were used in the analyses.

Chemiluminescence immunoassay

MAA–LDL was produced as previously described (35). Serum and saliva IgM and IgG Ab levels to MAA–LDL and serum IgM and IgG Ab levels to phosphocholine-modified keyhole limpet hemocyanin (Biosearch Technologies, Petaluma, CA) were measured by chemiluminescence immunoassay as previously described (36, 37). MicroFluor plates (Thermo Fisher Scientific) were coated with 5 μg/ml Ags dissolved in PBS–EDTA and incubated overnight at 4°C. The plates were washed with PBS–EDTA three times with an automated plate washer. Plates were blocked with 0.5% fish gelatin (Fg) in PBS–EDTA and incubated at room temperature for one hour. Serum and saliva samples, standards, and controls were diluted with 0.5% fish gelatin–PBS–EDTA. Samples were diluted 1:25 (IgA), 1:10 (IgM), and 1:10 (IgG) for saliva and 1:100 (IgA), 1:1000 (IgM), and 1:10,000 (IgG) for serum samples. The Ab levels were measured with the following alkaline phosphatase-labeled Abs: anti-human IgA-ALP (A9669; Sigma-Aldrich), anti-human IgM-ALP (A9794; Sigma-Aldrich), and anti-human IgG-ALP (A3187; Sigma-Aldrich). The chemiluminescence was detected using Lumi-Phos 530 substrate (Lumigen) and a Victor 3 Multilabel Counter (PerkinElmer/Wallac). Duplicate measurements were performed for each sample. Results are represented in relative light units (RLU) per 100 ms. For total serum and saliva IgA, IgM, and IgG Ab level measurements, anti-human IgA (I0884; Sigma-Aldrich), anti-human IgM (I0759; Sigma-Aldrich), and anti-human IgG (I3382; Sigma-Aldrich) polyclonal Abs produced in goat were used as capture Abs. The aforementioned alkaline phosphatase-labeled Abs were used as detection Abs. Total Ab concentrations were calculated from a standard curve, which was added to each plate. Results are represented in milligrams per milliliter for serum and micrograms per milliliter for saliva samples.

Oral microbiota analysis

DNA extraction and sequencing. Total DNA was extracted from resting saliva samples using a PowerSoil DNA Isolation kit (QIAGEN, Hilden, Germany) following the National Institutes of Health Human Microbiome Project guidelines (38). The DNA concentration was assessed using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

All library preparation and sequencing work was performed at the Institute for Molecular Medicine Finland (FIMM;

University of Helsinki) (http://www.fimm.fi). Briefly, the V4 region of the 16S rRNA gene was amplified using the original 515F/806R primer pair (39). Amplification was done in a multiplex PCR reaction with locus-specific primers carrying Illumina adapter tails and two Illumina P5/P7 index primers. PCR reaction conditions were as follows: initial denaturation at 98°C for 30 s; 30 cycles at 98°C for 10 s, at 56°C for 30 s, and at 72°C for 15 s; final extension at 72°C for 10 min. PCR products were pooled in equal volumes and purified with an Agencourt AMPure XP PCR purification kit (Beckman Coulter, Brea, CA), according to the manufacturer's instructions, using 0.8 × volume of beads compared with library pool volume. The final library was quantified using an Agilent High Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA) on an Agilent 2100 Bioanalyzer. Barcoded amplicons were sequenced on an Illumina MiSeq platform to provide paired-end 250-bp reads by FIMM.

Read data processing. Sequence data were demultiplexed by FIMM. Adapter sequences were removed in paired-end mode using Cutadapt v.2.8 (40). After this step, a total of 2,032,686 reads that represented an average of 81,307 reads per sample (range = 24,715–134,148) were processed using the R package DADA2 (41) implemented in QIIME2 v.2020.2 (42). Primer sequences were trimmed from the 5′ end of all reads, and reads were truncated at the 3′ end to control for data quality (forward reads at 212 bp and reverse reads at 200 bp). Read data were denoised and dereplicated, and putative chimeric sequences were removed using default parameters in DADA2 (41). These procedures left 1,245,177 sequences that represented 1,041 amplicon sequence variants (ASVs) over all samples. The naive Bayes classifier, pretrained on the SILVA reference database v.132 (43), was used to assign bacterial taxonomy to ASVs (44). Low-abundance (<10 reads across all samples) ASVs were removed, and the filtered feature table was rarefied to 17,035 reads per sample to minimize biases caused by variation in sequencing depth among samples (45). The final data for oral microbiota analysis represented 623 ASVs (average of 134 ASVs per sample; range = 74–195 ASVs per sample).

Statistical analyses

Statistical analyses of baseline data characteristics and data related to immunoassays were performed by IBM SPSS Statistics 26. A Wilcoxon–Mann–Whitney nonparametric test was used for comparison of variables. The p values < 0.05 were considered significant.

Statistical analyses related to microbiota were performed using R v.4.0.2 (46), unless otherwise stated. The oral microbiota α-diversity was estimated by calculating the number of observed ASVs, Shannon index, and Faith phylogenetic diversity index for each subject. Significant differences in α-diversity estimates were determined using the Wilcoxon rank-sum tests. Differences in β-diversity between samples were visualized by principal coordinate analysis, using Emperor (47), based on Bray–Curtis dissimilarities and unweighted and weighted UniFrac distances between samples. The permutational ANOVA (PERMANOVA; 999 permutations) implemented by the adonis function in the R package VEGAN (48) was used to assess statistical significance of sample grouping. Potential differences in dispersion between study groups were quantified with the dispersion test implemented by the PERMDISP in QIIME2 (42, 49). To quantify differences in the oral microbial community composition, we performed the Wilcoxon rank-sum tests, comparing relative abundance of key bacterial species (the top 10 in abundance account for >70% of the community) between the IgA-deficient and control subjects. We used the permuted mean difference tests operating at the level of individual ASVs, with 10,000 permutations and discrete false discovery rate correction at an α of 0.1 using DS-FDR v.0.0.2 (50) to detect differentially represented taxa between study groups. We also tested for potential associations between continuous metadata variables, α-diversity estimates, and taxa relative abundance by computing a correlation matrix based on Pearson correlation coefficients using the R package ggcorrplot v.0.1.3.

Data availability statement

NFBC1966 data are available from the University of Oulu, Infrastructure for Population Studies. Permission to use the data can be requested for research purposes via the electronic material request portal. In the use of data, NFBC1966 follows the European Union General Data Protection Regulation (679/2016) and Finnish Data Protection Act. The use of personal data is based on cohort participant's written informed consent at his/her latest follow-up study, which may cause limitations to its use. Please, contact NFBC project center (http://NFBCprojectcenter@oulu.fi) and visit the cohort Web site (http://www.oulu.fi/nfbc) for more information.

RESULTS

Baseline data

Baseline characteristics of the study are represented in Table I. The serum total IgA Ab levels varied between 0.00 and 0.04 mg/ml in subjects with SIgAD and between 2.00 and 4.93 mg/ml in control subjects. The salivary IgA concentration was 0.00 μg/ml in each of the five subjects with IgA deficiency, whereas in control subjects, the salivary IgA levels varied between 31.42 and 216.95 μg/ml. There were no statistically significant differences in body composition, lipid profile, blood pressure, or blood cell concentrations between groups, although the subjects with IgA deficiency seemed to be slightly heavier compared with control subjects ($p = 0.192$). In OGTT, the glucose concentration at 30 min was higher in subjects with IgA deficiency ($p = 0.023$), although there were no significant differences at 0 min, 60 min, or at 120 min.

Total serum and saliva IgM and IgG Ab levels

Serum. There was no difference in total serum IgM Ab levels between the IgA-deficiency group and the control group. The mean total serum IgM Ab level was 1.47 mg/ml in subjects

TABLE I. Baseline characteristics of the study

Variable	IgA Deficiency (n = 5)	Control Group (n = 20)	p Value
Gender			
Male/female (n)	3:2	8:12	
IgA			
Serum total IgA (mg/ml)	0.017 (0.00–0.04)	3.18 (2.00–4.93)	0.0002
Saliva total IgA (μg/ml)	0.00 (0.00–0.00)	100.18 (31.42–216.95)	0.0002
Body composition			
Height (cm)	174.8 (158.6–188.3)	171.8 (160.5–187.3)	0.530
Weight (kg)	85.42 (59.30–100.50)	72.38 (55.60–98.60)	0.192
BMI (kg/m^2)	28.37 (20.85–40.00)	24.47 (19.30–32.65)	0.303
Lipid profile, metabolic, and hematological values			
Serum LDL (mmol/l)	3.52 (2.40–4.40)	3.66 (2.40–5.40)	0.668
Serum triglycerides (mmol/l)	1.52 (0.68–3.22)	1.12 (0.54–3.10)	0.530
Fasting plasma glucose (mmol/l)	5.5 (4.8–5.8)	5.3 (4.7–6.5)	0.148
Plasma glucose, 0 min OGTT (mmol/l)	5.5 (4.8–5.8)[a]	5.4 (4.7–6.7)	0.148
Plasma glucose, 30 min OGTT (mmol/l)	9.6 (8.2–10.7)[a]	7.9 (6.3–9.8)	0.023
Plasma glucose, 60 min OGTT (mmol/l)	7.5 (3.6–10.9)[a]	6.9 (4.8–13.3)	0.477
Plasma glucose, 120 min OGTT (mmol/l)	5.3 (3.3–8.6)[a]	5.5 (3.6–7.2)	0.575
Hemoglobin (g/l)	144.60 (132.00–151.00)	138.20 (114.00–163.00)	0.336
Leukocytes (1×10^9/l)	5.74 (2.80–10.60)	5.43 (3.50–8.10)	0.767
Blood pressure			
Systolic pressure (mmHg)	121 (109–139)	123 (98–160)	0.921
Diastolic pressure (mmHg)	82 (76–87)	81 (65–98)	0.767

Values are reported as mean (range) unless otherwise indicated. Statistical significance was tested with Mann–Whitney U test.
[a]n = 4.

with IgA deficiency and 1.42 mg/ml in control group (p = 0.717). Also, total IgG Ab levels were similar between subjects with and without SIgAD. The mean total serum IgG Ab level was 17.10 mg/ml in subjects with IgA deficiency and 17.30 mg/ml in control subjects (p = 0.947).

Saliva. In saliva, the total IgM Ab levels were significantly higher in subjects with IgA deficiency when compared with control subjects. The mean total IgM Ab level was 5.79 μg/ml in subjects with IgA deficiency and 1.52 μg/ml in control subjects (p = 0.003) (Fig. 1C). The IgG total Ab levels in saliva did not differ between groups. The mean total IgG Ab level was 6.65 μg/ml in subjects with IgA deficiency and 5.30 μg/ml in control subjects (p = 0.371) (Fig. 1C).

Natural Ab levels to oxidized epitopes

Serum IgM and IgG to MAA–LDL. In serum, the IgG Ab levels to MAA–LDL were significantly higher in subjects with IgA deficiency. The mean serum IgG to MAA–LDL was 47002 RLU/100 ms in subjects with IgA deficiency, and 26953 RLU/100 ms in control subjects (p = 0.012) (Fig. 1A). The serum IgM Ab levels to MAA–LDL were slightly higher in subjects with IgA deficiency, although the difference was not statistically significant. The mean serum IgM Ab to MAA–LDL was 43399 RLU/100 ms in subjects with IgA deficiency and 34602 RLU/100 ms in control subjects (p = 0.575) (Fig. 1A).

Serum IgM and IgG to phosphocholine. There were no significant differences between serum IgM or IgG Ab levels to phosphocholine. The mean serum IgM Ab level to phosphocholine was 19290 RLU/100 ms in subjects with IgA deficiency and 13891 RLU/100 ms in control subjects (p = 0.921) (Fig. 1B). The mean serum IgG Ab to phosphocholine was 13095 RLU/100 ms in subjects with IgA deficiency and 14487 RLU/100 ms in control subjects (p = 0.818) (Fig. 1B).

Saliva IgM and IgG to MAA–LDL. In saliva, both IgM and IgG Ab levels to MAA–LDL were significantly higher in subjects with IgA deficiency. The mean IgM to MAA–LDL was 42086 RLU/100 ms in subjects with IgA deficiency and 7552 RLU/100 ms in control subjects (p = 0.00015) (Fig. 1D). The mean IgG to MAA–LDL was 14861 RLU/100 ms in subjects with IgA deficiency and 7418 RLU/100 ms in control subjects (p = 0.015) (Fig. 1D).

Oral microbiota

We used high-throughput 16S rRNA gene sequencing to characterize the oral microbiota in humans with or without SIgAD. We identified 623 ASVs from 10 bacterial phyla. Four dominant phyla accounted for > 92% of the oral microbiota community: Firmicutes (mean = 44%), Bacteroidetes (22%), Proteobacteria (16%), and Actinobacteria (10%) (Supplemental Table I). The six bacterial phyla detected at comparatively low levels were Fusobacteria, Patescibacteria, Epsilonbacteraeota, Spirochaetes, Synergistetes, and Tenericutes. Firmicutes were dominated by members of the *Veillonellaceae* and *Streptococcaceae* families (mean = 23% and 15% of total community, respectively), Bacteroidetes were dominated by *Prevotellaceae* (20%), whereas members of the *Pasteurellaceae* were the most abundant in Proteobacteria (10% of total community) and the *Micrococcaceae* family (7%) in Actinobacteria phylum (Supplemental Table I). Only three bacterial genera together

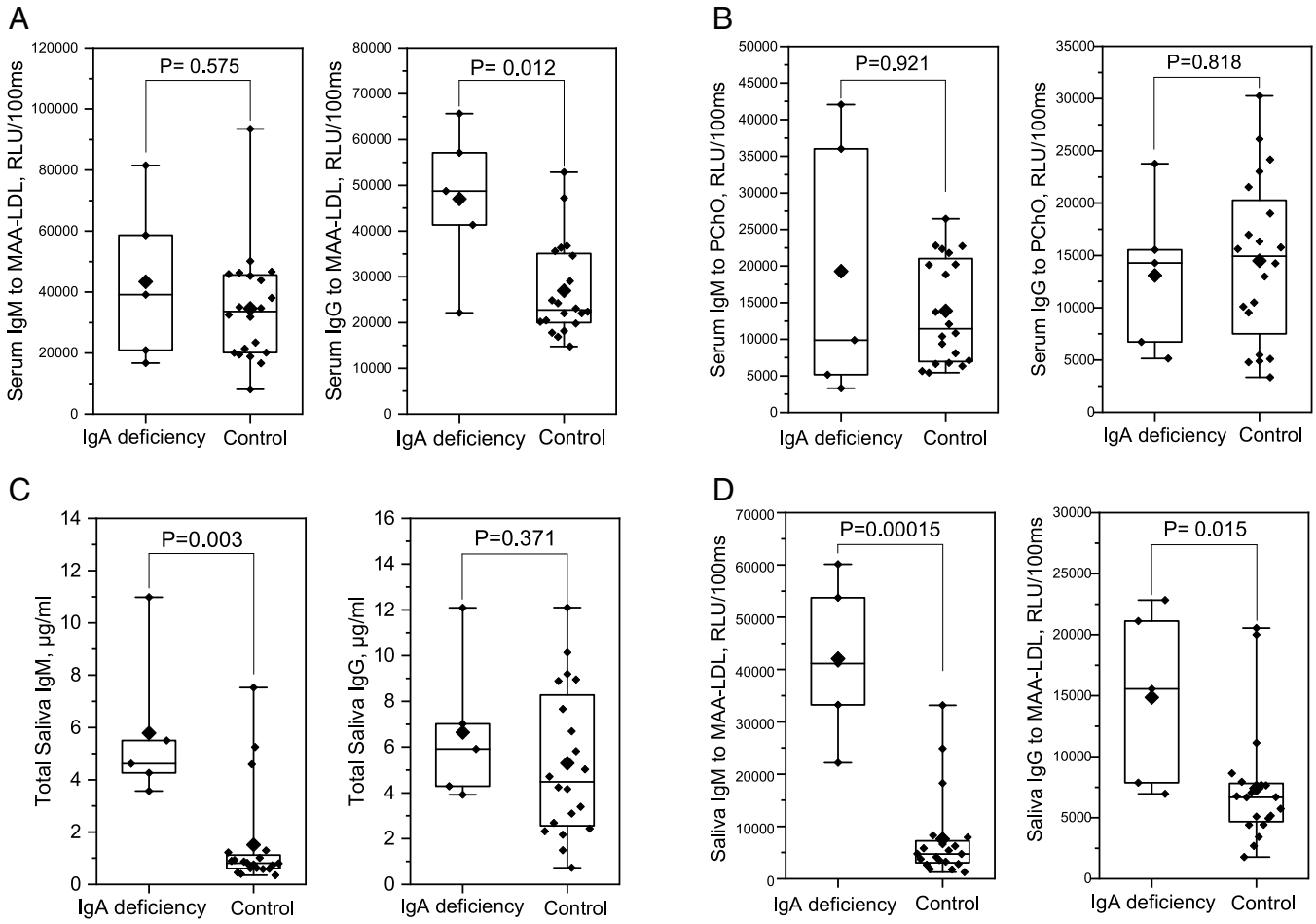

FIGURE 1. Levels of saliva and serum IgM and IgG Abs in subjects with and without SIgAD.

(**A**) Levels of serum IgM and IgG Ab to MAA-LDL. (**B**) Levels of serum IgM and IgG Ab to phosphocholine. (**C**) Total saliva levels of IgM and IgG Ab. (**D**) Levels of saliva IgM and IgG Ab to MAA-LDL. The small black diamonds represent individual Ab levels. The big black diamonds represent the mean value.

accounted for an almost half (>48%) of the oral microbiota community: *Veillonella*, *Streptococcus*, and *Prevotella* (Fig. 2A). This community composition is typical for oral microbiota of healthy adult humans (25, 51).

Variation in oral microbiota α-and β-diversity. The oral microbiota α-diversity did not differ significantly ($p > 0.05$, Wilcoxon rank-sum test) between samples from subjects with or without SIgAD (Fig. 2B). We found no significant associations between α-diversity estimates and levels of the total IgA in serum samples ($p > 0.05$, Pearson correlations). These patterns were consistent across all three α-diversity metrics used (e.g., number of observed ASVs, Shannon index, and Faith phylogenetic diversity index). We also observed no significant difference in α-diversity estimates between male and female subjects ($p > 0.05$, Wilcoxon rank-sum test). In our exploratory analysis, we found that, except for a weak positive correlation ($r = 0.4$, $p < 0.05$, Pearson correlation) between the community richness (number of observed ASVs) and saliva total IgG levels, neither of the other immunological parameters nor host body composition measures, lipid profile, metabolic, and hematological values were significantly correlated with the oral microbiota α-diversity ($p > 0.05$, Pearson correlations; Supplemental Fig. 1).

Subjects with and without SIgAD exhibit similar oral microbiota profiles, with no apparent sample clustering between study groups (Fig. 2C, Supplemental Fig. 2). Irrespective of the distance metric used, we observed no significant differences in β-diversity between IgA-deficiency and control group ($p > 0.05$, PERMANOVA based on Bray–Curtis dissimilarity, unweighted and weighted UniFrac distances; see Supplemental Table II). We also found no significant differences in dispersion between IgA-deficiency and control groups ($p > 0.05$, PERMDISP), a signature commonly associated with dysbiotic microbiota in various host conditions, including some forms of immunodeficiency (52). Similar to α-diversity, host gender, immunological parameters, and most other baseline characteristics of the study population had little notable effect on oral

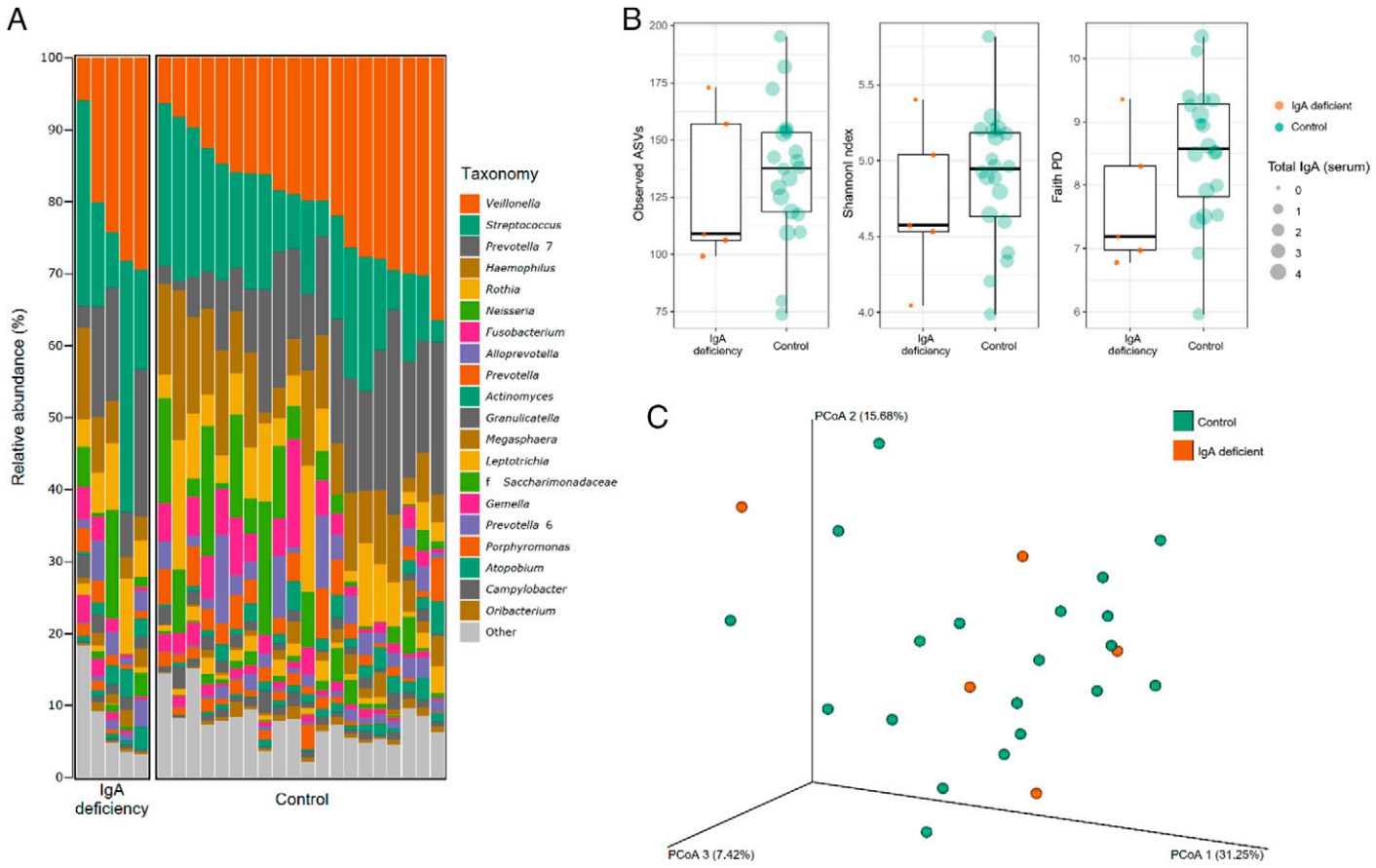

FIGURE 2. Oral microbiota in subjects with or without SIgAD.

(**A**) Relative abundance of bacterial taxa (at the genus level) in the oral microbiota of subjects with or without SIgAD. The community composition for individual samples grouped according to the IgA-deficiency status is shown (e.g., "IgA deficiency" and "Control," respectively). Taxa are sorted according to the relative abundance (taxa with the relative abundance of <1%, collectively referred to as "Other"). (**B**) Measures of the α-diversity for the oral microbiota of subjects with or without SIgAD (e.g., "IgA deficiency" and "Control," respectively). Box and whisker plots represent the median and interquartile range of α-diversity estimates (i.e., number of observed ASVs, Shannon index, Faith Phylogenetic diversity index [PD]), whereas size of the points corresponds to level (mg/ml) of the total IgA in serum samples. All comparisons were nonsignificant. $p > 0.05$, Wilcoxon rank-sum test. (**C**) Differences in the oral microbiota profiles of subjects with or without SIgAD (e.g., "IgA deficient" and "Control," respectively). Principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity between the oral microbiota profiles of IgA-deficient and control subjects. Each point represents a single sample; color indicates the study group.

microbiota β-diversity ($p > 0.05$, PERMANOVA; Supplemental Table II). However, subject weight, body mass index (BMI), and fasting plasma glucose levels had a statistically significant explanatory effect on the oral microbiota structure ($p < 0.05$, PERMANOVA, for Bray–Curtis dissimilarity, and weighted UniFrac; Supplemental Table II). The implication is that variation in oral microbiota profiles among study subjects is better explained by metabolic parameters and host body composition rather than IgA-deficiency status.

Variation in oral microbiota composition. The oral microbiota of subjects with and without SIgAD were characterized by similar community composition. We found no significant differences in the relative abundance of key bacterial species (top 10 in abundance account for > 70% of the community) between the IgA-deficient and control subjects ($p > 0.05$, Wilcoxon rank-sum test; Fig. 3). These taxa include *Veillonella* sp., *Streptococcus* sp., *Haemophilus* sp., *Prevotella melaninogenica*, *Neisseria* sp., *Prevotella* 7 sp., *Rothia* sp., *Fusobacterium* sp., *Alloprevotella* sp. and *Actinomyces* sp., which were characterized by considerable interindividual variation in the relative abundance, albeit similar distribution in both study groups (Fig. 3). This pattern was further reinforced by the permuted mean difference tests that operate at the level of individual bacterial ASVs rather than taxonomic groups (50), as none of the ASVs were found to be differentially represented between IgA-deficiency and control group ($p > 0.05$, permutation test with discrete false discovery rate correction). When comparing continuous metadata variables, the level of total IgA Ab in serum of the study subjects was negatively correlated with the relative

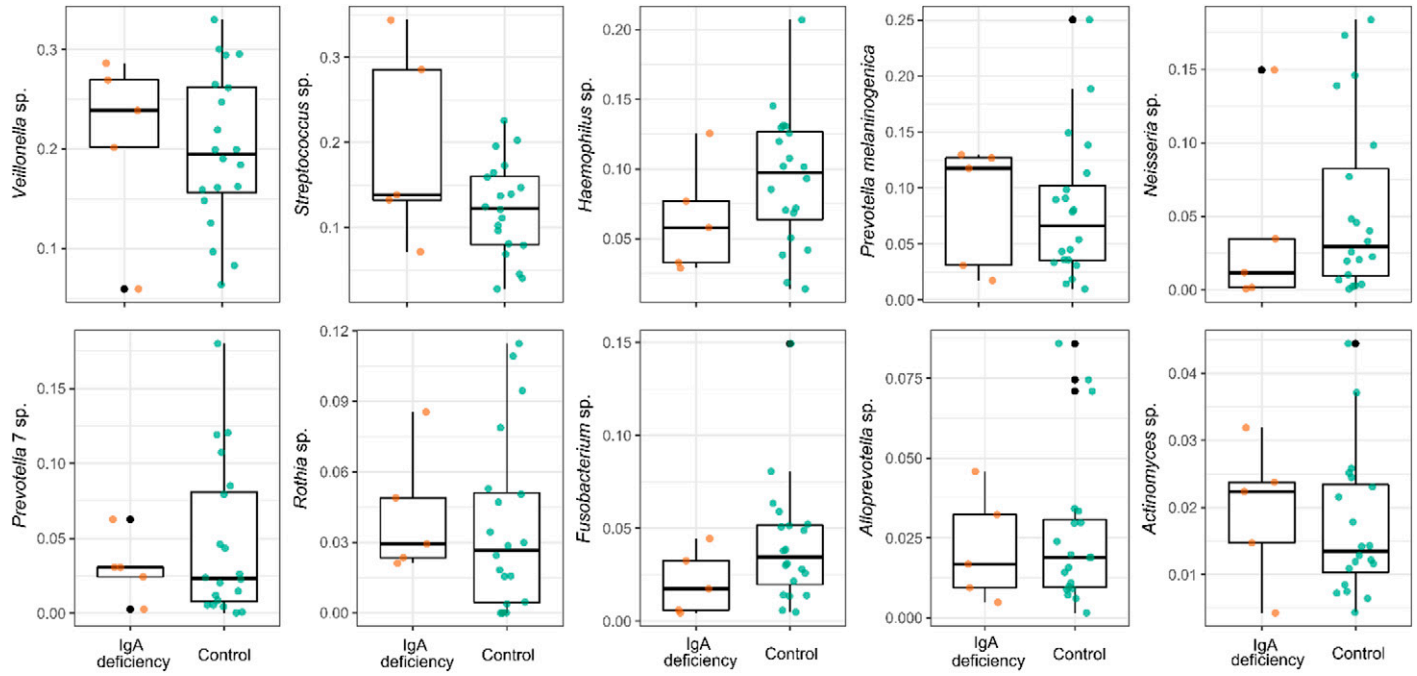

FIGURE 3. Relative abundance of bacterial species in the oral microbial community of the subjects with or without SIgAD.

The relative abundance of key bacterial species are shown (top 10 in abundance account for >70% of the community). Box and whisker plots represent the median and interquartile range of taxa relative abundance, whereas the color of the points indicate IgA-deficiency status (e.g., "IgA deficiency" and "Control"). All comparisons were nonsignificant. $p > 0.05$, Wilcoxon rank-sum test.

abundance of bacteria from *Streptococcus* genus, although such association was only marginally significant ($r = -0.4$; $p = 0.042$, Pearson correlation). Several other associations that were not related to IgA deficiency but were potentially worth further exploration include the significant positive correlation between *Veillonella* and fasting plasma glucose levels (but compare the negative association with *Fusobacterium*), subject weight, and BMI ($p < 0.05$, Pearson correlation; Supplemental Fig. 1), and these were the only variables that had significant explanatory effect on the oral microbiota β-diversity (Supplemental Table II). Note, however, that weight and BMI are both strongly positively associated with the fasting plasma glucose levels ($r=0.7$; $p < 0.05$, Pearson correlation). Taken together, our data suggest that IgA deficiency, as such, does not manifest in altered oral microbiota community composition in humans.

DISCUSSION

In the current study, we analyzed natural Abs to oxidized epitopes from saliva and serum of subjects with and without SIgAD. Our observation was that the levels of IgM and IgG Abs to MAA–LDL were markedly higher in subjects with SIgAD compared with controls. We also compared serum IgM and IgG Ab levels to phosphocholine between subjects with and without SIgAD, but no significant differences were observed between groups. MAA–LDL and phosphocholine were chosen as Ags in this study to likely detect natural Abs. As IgM to MAA–LDL and phosphocholine can be considered as natural Abs, IgG to MAA–LDL and phosphocholine, however, are presumably a pool of mixed natural and adaptive Abs. In addition to Abs, we used 16S rRNA gene sequencing to characterize the oral microbiota in subjects with or without SIgAD to investigate whether subjects with SIgAD have altered oral microbiota. We found no statistically significant differences in the oral microbiota diversity and composition between subjects with SIgAD and controls.

Although IgA is the second most prevalent Ig in serum after IgG, its role in serum is poorly understood (53). Although serum IgA does not activate the classical way of complement, it has been suggested that serum IgA activates the phagocytosis of pathogens via a certain IgA Fc receptor (FcαRI) on phagocytes (9, 10). In the current study, subjects with IgA deficiency had no difference in total serum IgM and IgG levels compared with healthy controls, implying that the lack of serum IgA does not itself provoke a compensatory elevation of serum IgM or IgG. In saliva and other secretions, IgA is called secretory IgA (54) and has a more obvious role. Secretory IgA is considered the first-line humoral defense against mucosal pathogens, in which it inhibits bacterial adherence and limits foreign Ags from penetrating the mucosa (29). The absence of secretory IgA in SIgAD is often compensated with elevation in production of secretory IgM (10, 53, 55), which is consistent with the higher saliva total IgM level in subjects with SIgAD in our study. Some studies have also reported a compensatory

elevation in the salivary IgG levels of patients with selective IgA deficiency (17–19). In our study, the total salivary IgG levels were similar between subjects with and without SIgAD.

Abs can be divided into natural and adaptive Abs. Natural Abs are characterized by low affinity and broad reactivity to self-antigens (3). They consist mainly of IgM isotype and are produced by a subpopulation of B cells prior to an encounter with pathogens or foreign Ags (2). Natural IgM Abs improve phagocytosis of apoptotic cells by identifying those cells after, for example, oxidative changes (2). Natural Abs are also known to recognize and bind oxidized low-density LDLs (2). Lipid peroxidation-derived MDA and MAA epitopes are major targets of natural IgM Abs (56), and even in newborns, natural IgM Abs have been shown to already recognize MAA–LDL (8). In this study, we used MAA–LDL as an Ag in an aim to detect natural Abs. Another Ag we used was phosphocholine, an oxidized neodeterminant known to be recognized by IgM natural Abs and which is exposed in lipid oxidation and cell apoptosis and found in bacteria such as *S. pneumoniae* (20, 57). No significant differences in IgM or IgG Abs to phosphocholine were observed in the current study. This result is in contrary to a previous study comparing SIgAD patients and healthy controls, in which an enhancement in IgG Ab reactivity to phosphorylcholine was found, and a compensatory role for IgG in defense from infection in IgA deficiency was suggested (20).

In the current study, the saliva IgM natural Abs to MAA–LDL were markedly elevated in SIgAD patients compared with controls. In serum, the IgM Abs to MAA–LDL were also slightly elevated in SIgAD patients, but the difference was not statistically significant. Compensatory production of secretory IgM in SIgAD patients can be assumed to at least partially explain higher levels of natural saliva IgM Abs to MAA–LDL. To our knowledge, this is the first study examining natural Abs to MAA–LDL in SIgAD patients. Previously, we showed that healthy subjects have significant amounts of IgA and IgG Abs to MAA–LDL in saliva, and those Abs also showed cross-reactive binding to a periodontal pathogen, *Porphyromonas gingivalis* (37). In that study, saliva IgM Ab levels to MAA–LDL were minimal in healthy subjects.

Natural Abs consist mainly of IgM isotypes, which are also the most studied natural Ab, but there are also natural IgA and IgG Abs in the circulation of healthy individuals (3, 58, 59). In contrast to natural IgM, natural IgG-producing B cells are inactive at birth, and it can take more than two years before natural IgG Abs are at a detectable level in serum (60). Natural IgG Abs are shown to have a fundamental role in innate immune responses. In vitro studies have shown that purified natural IgG from uninfected human serum engages with serum lectins to recognize Gram-negative and Gram-positive bacteria and enhance the phagocytosis of these bacteria (58). Besides protection from pathogens, natural IgG Abs also participate in regulation of inflammatory conditions. Natural IgG Abs to MDA–LDL have been found to show high expression only in patients with inflammatory diseases, such as systemic lupus erythematosus, whereas natural IgG Abs to phosphorylcholine are present even in healthy people (2, 61). In the current study, the IgG Abs to MAA–LDL were significantly elevated in subjects with SIgAD in both saliva and serum. Because the total IgG levels in saliva and serum were similar between IgA deficiency and healthy subjects, it remains unclear whether the elevation of saliva and serum IgG levels to MAA–LDL is a compensation mechanism for the lack of IgA or whether it is because of some other cause, such as a systemic condition that is overrepresented among SIgAD patients.

The association of natural Abs to oxidized LDL and systemic diseases, especially atherosclerosis, has been investigated in several studies, although focusing more in MDA–LDL over MAA–LDL. In the matter of natural IgM Ab, circulating IgM autoantibodies to MDA–LDL have been found to be inversely linked to atherosclerosis (62, 63). Association of IgG to MDA–LDL or MAA–LDL and atherosclerosis are less definitive (62, 64). However, our previous data show that low levels of plasma IgG to MDA–LDL are associated with type 2 diabetes as an independent risk factor (65). More studies of the role of serum and natural salivary Abs to MAA–LDL in cardiovascular and other diseases are needed if we are to understand the consequences of the elevated natural Ab levels.

We previously showed that salivary IgA to MAA–LDL is associated with coronary disease (66). Also, IgA Abs to phosphocholine are associated with cardiovascular disease risk (67). However, there is a lack of studies investigating the prevalence of cardiovascular disease among people with IgA deficiency. Some autoimmune diseases, such as type 1 diabetes mellitus, are more common in SIgAD patients (11, 13). In our study, the glucose concentration in OGTT at 30 min was higher in subjects with SIgAD, although there was no difference in fasting plasma glucose levels (Table I). Further studies are needed to replicate this observation.

In addition to natural Abs, we compared the oral microbiota of the subjects with and without SIgAD. We found no significant differences in α-diversity, β-diversity, dispersion, or community composition between IgA-deficient and control subjects. We further investigated whether the variation in oral microbiota profiles of the study subjects could be explained by IgM or IgG Ab levels instead of IgA, or alternatively, by gender, host body composition measures, lipid profile, or metabolic or hematological values. Regarding Ig parameters, we only found a weak positive correlation between the community richness and total saliva IgG. However, subject body mass, BMI, and fasting plasma glucose levels had statistically significant associations with oral microbiota structure, likely due to variation in the relative abundance of *Veillonella* spp. and *Fusobacterium* spp. Our results suggest that IgA deficiency itself does not manifest in altered oral microbiota community composition or impact community diversity (Fig. 2). Rather, the variation in oral microbiota profiles of the study subjects is better explained by metabolic parameters and host body composition.

The relationship between IgA and microbiota has been studied in recent years, focusing on the gut microbiota. Secretory IgA is known to be involved in host–microbiota

interactions at intestinal mucosal surfaces, where IgA extensively coats the bacteria residing in the gut. IgA responses to microbiota develop through T cell–independent and T cell–dependent pathways and target a taxonomically distinct subpopulation of the microbiota, which include noninvasive commensals, penetrant commensals, and invasive pathogens (68, 69). Recognition of these bacteria are suggested to involve both high-affinity IgA from the T cell–dependent pathway (68) and low-affinity, polyreactive IgA of the T cell–independent pathway (68, 69). The mechanisms by which secretory IgA drives host–microbiota interactions are not fully understood, yet even by exclusion of certain secretory IgA-coated bacteria, secretory IgA can promote the growth of beneficial commensals (68). Thus, secretory IgA can shape the composition and invasiveness of intestinal bacteria.

Secretory IgM is not as stable in its structure as secretory IgA, but it is suggested to provide mucosal protection in early life, before the production of secretory IgA (68). In the lack of IgA, secretory IgM was shown to partially restore the intestinal microbiota diversity (68). Contrary to our results on oral microbiota, recent publications have demonstrated changes, such as mild intestinal dysbiosis (31), changes in relative abundancies of certain taxa (30, 31), and decrease in α-diversity (30, 32), in the intestinal microbiota of SIgAD patients.

There is a lack of previous studies investigating oral microbiota in SIgAD patients. However, oropharyngeal microbiota of patients with CVID, an immunodeficiency disorder in which there is also a deficiency of IgG and/or IgM in addition to deficiency of IgA, was investigated in a recent study. In that work, associations between serum IgA, oropharyngeal microbiota, and the severity of lung diseases, were studied (70). Contrary to the previous studies of IgA deficiency or CVID and gut microbiota, an increase in α-diversity in the oropharyngeal microbiota was found to be associated with low levels of serum IgA in CVID patients (70).

Although clear alterations in the gut microbiota of SIgAD patients have been described in the recent studies, our data suggest that oral microbiota is not affected as much by the lack of IgA, as the compensatory effects of increased secretory IgM and possibly IgG may be sufficient to preserve the oral microbiota. In contrast to IgM and IgG, IgA Ab is a poor activator of the complement system and is considered to have more of an anti-inflammatory role (69, 71). In the intestine, where bacterial loads increase to high densities toward the ileum and colon, IgA has an important role in the immune protection, which IgA conducts in a noninflammatory manner (72). The largest population of IgA-producing plasma cells are located in the intestine, whereas only minor populations are detectable in extraintestinal tissues. Also, the primary sites of IgA induction are the gut-associated lymph tissues, especially Peyer patches in the intestine (69). Thus, in the intestine, the role of IgA is possibly more important, and lowered IgA functions possibly have more impact, as secretory IgM- and IgG-mediated compensatory responses induce bystander inflammation that potentially affects the gut microbiota and leads to dysbiosis. Another possible explanation, in contrast, for why no dysbiosis was detected in the oral microbiota of subjects with SIgAD, could be the nature of the oral microbiome. The oral biogeography is more complex and is inhabited by microbial communities of site specialists. These heterogenous and structurally highly organized oral biofilms (23, 73, 74) are also potentially more resistant to selective stresses, such as Ab-mediated control by the host.

A great advantage in this study is the powerful NFBC1966 study cohort data. The study cohort is large and controlled, the subjects are matched in age and nationality, and they are not recruited from a hospital environment. These factors offer an advantage over analyzes and comparisons made from more heterogenous study populations. Investigating natural Abs and oral microbiota of subjects with SIgAD is challenging because of the generally low frequency of IgA deficiency, and a limit of our study is the low number of IgA-deficient patients. However, this unique cohort adds reliability to the results. Future studies using an independent cohort with a larger number of IgA-deficient subjects and a longitudinal design are needed to confirm our results.

In conclusion, in this study with five SIgAD patients and twenty controls from northern Finnish birth cohort, we discovered elevated levels of Abs to MAA–LDL in saliva and serum of SIgAD patients, including natural secretory IgM Abs and serum and saliva IgG Abs. Also, our data on oral microbiota suggest that SIgAD patients have no significant alterations in their oral microbiota diversity and composition compared with subjects without SIgAD. Our findings support the observation that SIgAD causes activation of compensation mechanisms, such as elevation of secretory IgM Abs that enables SIgAD patients to remain healthy. To our knowledge, this is the first study investigating natural Abs to MAA–LDL and oral microbiota in subjects with SIgAD.

DISCLOSURES

The authors have no financial conflicts of interest.

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