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# Prevalence of saliva immunoglobulin A antibodies reactive with severe acute respiratory syndrome coronavirus 2 among Japanese people unexposed to the virus

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## Abstract

While the COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses a threat to public health as the number of cases and COVID-19-related deaths are increasing worldwide, the incidence of the virus infection is extremely low in Japan compared with many other countries. To explain this uncommon phenomenon, we investigated the prevalence of naturally occurring ("natural") antibodies, focusing on those of the secretory immunoglobulin A (sIgA) form, reactive with SARS-CoV-2 among Japanese people. One hundred and eighty healthy Japanese volunteers of a wide range of age who had been considered to be unexposed to SARS-CoV-2 participated in this study. Saliva samples and blood samples were collected from all of the 180 participants and 139 adults (aged ≥ 20 years) included therein, respectively. The determination of saliva IgA antibodies, mostly comprising sIgA antibodies, as well as serum IgA and immunoglobulin G antibodies, reactive with the receptor binding domain of the SARS-CoV-2 spike-1 subunit proteins was conducted using an enzyme-linked immunosorbent assay. The major findings were that 52.78% (95% confidence interval, 45.21%–60.25%) of the

**Abbreviations:** ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; IgA, immunoglobulin A; IgG, immunoglobulin G; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sIgA, secretory immunoglobulin A; sIgAD, selective IgA deficiency.

individuals who had not been exposed to SARS-CoV-2 were positive for saliva IgA antibodies with a wide range of levels between 0.002 and 3.272 ng/mL, and that there may be a negative trend in positivity for the antibodies according to age. As we had expected, a frequent occurrence of assumable "natural" sIgA antibodies reactive with SARS-CoV-2 among the studied Japanese participant population was observed.

KEYWORDS
COVID-19, Japanese people, saliva IgA, SARS-CoV-2, secretory IgA, seroprevalence

## INTRODUCTION

In December 2019, a novel coronavirus termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of a new pandemic of severe acute respiratory syndrome known as COVID-19.[1] Since then, this emerging virus infection has spread globally with an unexpectedly high speed and has created a public health crisis, as well as major economic and social burdens. As of November 18, 2020, the ongoing pandemic of COVID-19 has infected more than 56 million people worldwide with the mean population-based infection rate (percentage of numbers of polymerase chain reaction [PCR]-confirmed cases per whole population) being 0.721%.[2] This is thought to be due to the highly efficient person-to-person transmission of SARS-CoV-2 and the lack of population-level immunity.[3] However, the incidence of COVID-19 varied markedly by geographical areas and nations. For example, infection rates as of November 1, 2020 for G7 countries comprising USA, France, UK, Italy, Germany, Canada, and Japan were estimated to be 2.802%, 2.176%, 1.524%, 1.173%, 0.651%, 0.638%, and 0.081%, respectively.[4] The data indicate that Japan remained one of the countries least severely affected by the ongoing COVID-19 pandemic owing to such an extremely low incidence of the disease, despite none of the Japanese people having undergone any vaccination for COVID-19. At this moment, there is no rational explanation for this uncommon phenomenon.

Several recent reports suggest that immunoglobulin G (IgG) responses and, to a greater extent, immunoglobulin A (IgA) responses to SARS-CoV-2 play a role in protecting the human host through neutralization of the virus infectivity.[5–9] In humans, the most common IgA form present in serum is a monomer, whereas at mucosal sites, IgA exists in mucosal secretions as polymeric molecules, foremost as dimeric IgA.[10] It is well accepted that such polymeric IgA constitutes a principal component of mucosal immunity and thus it is referred to as secretory IgA (sIgA) or mucosal IgA.

SARS-CoV-2 primarily infects the mucosal surfaces of the nasopharynx and the upper respiratory tract,[11] as well as the oral cavity,[12] at least until advanced stages of the disease (COVID-19) when viral RNA may become detectable in the circulation. The virus also infects the glands and mucosae of the oral cavity which harbor epithelial cells expressing angiotensin converting enzyme 2 (ACE2) and several other receptors for the virus spike proteins, particularly the receptor binding domain (RBD).[12,13] These findings also underscore a crucial role of sIgA antibodies in protecting mucosal surfaces against SARS-CoV-2 by neutralizing the virus and/or impeding its attachment to epithelial cells in the initial stage of the virus infection.

There is recent evidence showing that anti-SARS-CoV-2 immunity not only occurs after a natural infection, but may also precede such an active infection. Cross-reactivity to SARS-CoV-2 antigen peptides has been identified on T-cells and B-cells from prepandemic donors; S protein-reactive $CD4^+$ T-cells are not only detected in a large fraction of patients with COVID-19 but also in a smaller, but considerable, fraction of healthy individuals with no history of COVID-19.[14,15] Consistent with this, it was also demonstrated that antibodies, probably including polyreactive natural autoantibodies,[16] cross-reacting with SARS-CoV-2 have been detected in healthy individuals unexposed to the virus,[9,17,18] and that a cross-reactive human IgA monoclonal antibody, which binds to SARS-CoV-2 S proteins resulting in competitive inhibition of ACE2 receptor binding, has been developed successfully.[19]

All these findings led us to the hypothesis that lower population-based susceptibility to SARS-CoV-2 infection seen in Japan compared with G7 and many other countries is associated with a more frequent occurrence of sIgA antibodies reactive with the virus among Japanese people. Thus, as an initial step to approach this possibility, we implemented a cohort study with healthy Japanese participants unexposed to SARS-CoV-2, in which we measured the concentrations of the virus-reactive sIgA antibodies using saliva samples because sIgA antibodies, as secreted antibodies, are noninvasively accessible in saliva.[20,21] Measurements were also performed for SARS-CoV-2-reactive IgA and IgG concentrations in serum for comparison.

## MATERIAL AND METHODS

### Study approval and ethics

Approval to undertake the study was obtained from the Kanagawa Dental University Research Ethics Review Board (approval number: 792) on April 6, 2021. The study adhered to all the guidelines set forth in the Declaration of Helsinki (amended in October 2013), the Ethical Guidelines for Medical Research in Humans (noted by the Ministry of

Health, Labor and Welfare, the Japanese Government, in December 2014 [amended in February 2017]) and the Guidance of the Ethical Guidelines for Medical Research in Humans (enacted by the Ministry of Education and Science and the Ministry of Health, Labor and Welfare, the Japanese Government, in February 2015 [amended in March 2017]). Written informed consent was obtained from all participants themselves or informed assent from their parents (in the case of minor participants aged below 20 years), prior to the study onset.

The study was registered on April 1, 2021 in the database of the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR) that meets the JCMJE standards. The study ID was UMIN000043717.

## Study participants

This epidemiological humoral immunity survey was a cross-sectional study undertaken in the Kantoh District of Japan that is located in the middle area of the Main Island of the country constituting Metropolitan Tokyo and three neighboring Prefectures (Kanagawa, Chiba, and Saitama). This survey was conducted in two groups of individuals invited to participate in the present study. Group I volunteers aged between 20 and 75 years were recruited from the general public in the Kantoh District. Group II consisted of children and adolescents under treatment for their dental conditions, university students, and dentists aged between 3 and 71 years in Kanagawa Dental University Hospital, Yokosuka, Kanagawa, all of whom were inhabitants of the Kantoh District. The inclusion criteria were: to have Japanese racial background; to have had no history or experience of being diagnosed COVID-19, SARS-CoV-2 PCR-positive, nor COVID-19-related symptoms; and to have not experienced common cold-like symptoms during the preceding 2 weeks. Individuals were excluded if: they had received anti-SARS-CoV-2 vaccination; they were under treatment for systemic diseases or injuries; they had oral mucosal diseases with local bleeding; or they had participated in another clinical study within 1 month prior to the current study period.

## Study design and procedures

All eligible participants were requested to visit one of the following four study centers: Medical Station Clinic (Tokyo); Kanagawa Dental University Hospital (Kanagawa); Kanagawa Dental University Yokohama Clinic (Kanagawa); and Sato Dental Clinic (Kanagawa), between April 8, 2021 and May 8, 2021 for collection of both saliva and blood samples (in adult participants) and between May 1, 2021 and August 2, 2021 for collection of saliva samples only (in minor participants aged < 20 years). The actual number of saliva samples collected (with or without blood sample) from adult and minor participants every month during the indicated term for sample collection were: 138 and 0 in April; 1 and 19 in May, 0 and 4 in June; 0 and 13 in July; and 0 and 5 in August, 2021, respectively. All the participants were also instructed to refrain from taking any foods and drinks, as well as from tooth-brushing, for at least 1 hr before the collection of saliva samples.

Saliva samples were collected from all participants at the defined time period in the morning (9:30–10:30) to avoid diurnal variation of saliva flow. Sample collections were performed using Salivettes® or Salikids® (Sarstedt AG & Co., KG). The collected saliva samples were immediately centrifuged at 3000 × g for 5 min at 5°C. A portion of each sample was immediately subjected to a SARS-CoV-2 PCR test to confirm test-negative. Both saliva samples and serum samples stored at −80°C and −20°C, respectively, were transported to the immunology laboratory in Kanagawa Dental University where all samples were serologically assayed.

## Determination of SARS-CoV-2-reactive saliva and serum IgA antibodies and serum IgG antibodies

The detection and determination of IgA antibodies in saliva, as well as IgA and IgG antibodies in serum, reacting with the RBD of SARS-CoV-2 spike-1 subunit proteins, were performed using an enzyme-linked immunosorbent assay (ELISA) according to the previous report of Tsukinoki et al.[22] In this ELISA system, anti-IgA or anti-IgG specific antibodies are adsorbed on the plate as capture antibodies beforehand. For the measurement of SARS-CoV-2-reactive IgA antibodies in saliva mostly consisting of sIgA antibodies, and also serum IgA antibodies, spike-1-mFc recombinant protein (#40591-V05H1; Sino Biological) was used as the virus antigen. However, as this recombinant protein was found to nonspecifically bind, probably via its mFc moiety, to serum IgG antibodies, spike-1-His recombinant protein (#40591-V08B1; Sino Biological) was substituted for ELISA assays of their specific reactivity with SARS-CoV-2. Both antigens are SARS-CoV-2 spike-1 subunit proteins containing RBD. Biotin-labeling of these antigens was performed using a labeling kit (#BK01; Dojindo Laboratories) according to the manufacturer's instructions. Half-well ELISA plates added with saliva samples at 1:500 dilution or serum samples at 1:100 dilution in carbonate-bicarbonate buffer were incubated for 1 hr at 25°C, and then washed with washing buffer. To the wells thus coated with diluted saliva samples or serum samples, 100 µL per well of biotin-labeled antigen at a concentration of 1 µg/mL was added, and incubated for 1 hr at 25°C. The wells were washed five times with washing buffer, and then the plates were incubated with horseradish peroxidase (HRP) solution A (Bethyl Laboratories) for 1 hr at 25°C. The plates were developed by addition of the HRP substrate, TMB (Bethyl Laboratories), for 15 min at 25°C. Then the developing reaction was quenched by adding stop solution. The ODs were measured at 450 nm in a microplate absorbance reader (Bio-Rad Laboratories). As positive controls, two commercially available antibody products, that is, spike-neutralizing IgA antibody

(cat#E-AB-V1027; Elabscience; from 0 to 2 μg/mL) and spike-neutralizing IgG antibody (cat#40592-R001; Sino Biological; from 0 to 20 μg/mL) were used for the detection of SARS-CoV-2-reactive IgA and IgG, respectively. The positive control and negative control PBS were added to every assay plate for validation. The background absorbance value for the negative control was subtracted from the absorbance value for each saliva sample or serum sample to account for non-specific binding of biotin-labeled antigen to wells without antibody. The detection limits for saliva IgA, serum IgA, and serum IgG antibodies were 0.002, 0.163, and 1.0 ng/mL, respectively; thus, when the values observed were equal to or above the detection limits, the tests for each type of antibody were considered positive.

## Questionnaire

At saliva collection with or without blood drawing, the participants were asked to complete a questionnaire containing: current demographic characteristics (e.g., age, gender, body mass index, nativity, physical health, comorbidities such as pollinosis and other allergic disorders), oral care (particularly, daily frequency of tooth-brushing), history of vaccination for any viral diseases (e.g., influenza, type-B viral hepatitis, rubella, and mumps), current medication, and dietary habits including consumption of fermented foods and/or supplementary diets, as well as COVID-19-related symptoms.

## Statistics

Comparison of positivity for SARS-CoV-2-reactive immunoglobulins among different participant groups was analyzed using the chi-squared test. The correlation between saliva IgA, serum IgA, and serum IgG concentrations reactive to SARS-CoV-2 was evaluated by Pearson's correlation coefficient. Results with $P$ values $< 0.05$ were considered statistically significant. Analyses were performed using IBM SPSS Statistics version 27 (IBM).

# RESULTS

Consent to the present study was provided and the questionnaire was completed by 139 adult participants (aged ≥ 20 years) themselves or by each parent of 41 juvenile participants (aged < 20 years). Thus, both saliva and blood samples and only saliva samples were collected from all the enrolled adults and juveniles, respectively. None of the 180 participants had a clinical history or a current status consistent with COVID-19 and all tested negative for SARS-CoV-2 by RT-PCR.

All of the 180 individuals were tested for whether they had detectable amounts of SARS-CoV-2-reactive salivary IgA or not. The results showed that among them 95 individuals (52.78%) tested positive as shown in Table 1. The fraction that tested positive did not differ by gender, but varied by age; higher and lower positivities were observed for the youngest age group (<20 years) and the oldest age group (≥51 years), respectively, compared with the two intermediate age groups (20–35 years and 36–50 years groups). Thus, it was suggested that although not statistically significant, there was a negative trend of positivity for saliva IgA antibodies according to age. No association was found in positivity for saliva IgA antibodies with comorbidities, history of any vaccinations or daily oral care and/or dietary habits (data not shown).

We observed a broad range of the level of SARS-CoV-2-reactive saliva IgA antibodies with an over 1600-fold difference between the extremes (0.002 and 3.272 ng/mL) among the 95 test-positive individual samples. However, the

**TABLE 1** Proportion positive for saliva IgA antibodies reactive with SARS-CoV-2 in all participants of the study population stratified by gender and age groups

| Variable | Number of participants | Proportion of participants, % (95% CI)[a] | Positive for saliva IgA antibodies | | |
|---|---|---|---|---|---|
| | | | *N* | Proportion, % (95% CI)[a] | *P* value[b] |
| Overall | 180 | 100 | 95 | 52.78 (45.21–60.25) | |
| Gender | | | | | |
| Male | 96 | 53.33 (45.76–60.79) | 51 | 53.13 (42.66–63.39) | 1.000 |
| Female | 84 | 46.67 (39.21–54.24) | 44 | 52.38 (41.19–63.40) | |
| Age (years) | | | | | |
| <20 | 41 | 22.78 (16.87–29.61) | 27 | 65.85 (49.41–79.92) | 0.095 |
| 20–35 | 45 | 25.00 (18.86–31.99) | 23 | 51.11 (35.77–66.30) | |
| 36–50 | 42 | 23.33 (17.36–30.20) | 24 | 57.14 (40.96–72.28) | |
| ≥51 | 52 | 28.89 (22.39–36.10) | 21 | 40.38 (27.01–54.90) | |

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Estimated using exact binominal distribution.

[b]Estimated by $\chi^2$ test.

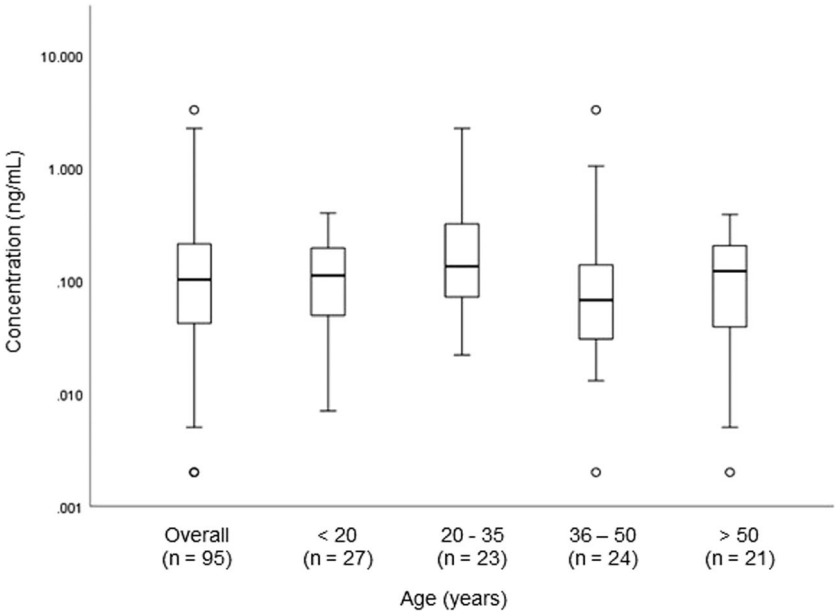


**FIGURE 1** Presence and levels of saliva immunoglobulin A antibodies reactive with severe acute respiratory syndrome coronavirus 2 in the overall participants and in the fractionated age groups of the study population. The box-plots indicate the medians (bold horizontal lines) and interquartile ranges (box boundaries), while the whiskers represent 1.5× interquartile ranges. The circles indicate measurements that are between 1.5 and 3 times the interquartile ranges.

results of 90 of the 95 samples (94.7%) clustered around a median of 0.102 ng/mL (Figure 1). Similar distribution patterns and medians of levels for test-positive samples were also observed in each age group; medians for <20 years-, 20–35 years-, 36–50 years-, and >50 years-groups were 0.111, 0.134, 0.067, and 0.121 ng/mL, respectively.

One hundred and thirty-nine adult participants provided matched saliva and serum samples collected during the same visit. All samples were tested by a multiplex assay for measuring the levels of SARS-CoV-2-reactive IgA antibodies in saliva, as well as those of equivalent IgA and IgG antibodies in serum. Sixty-eight (48.92%), 24 (17.27%), and 17 individuals (12.23%) were positive for SARS-CoV-2-reactive saliva IgA, serum IgA and serum IgG antibodies, respectively (Table 2). The fraction that tested positive for each immunoglobulin equivalent varied by age. It looks likely that although not statistically significant, there could be a negative trend of test positivity according to age for salivary IgA and serum IgG (Table 2).

We also conducted correlation analysis to learn possible correlation of the test positivity for saliva IgA antibodies with that for serum IgA and/or serum IgG antibodies. The results showed no significant correlation with the value for either serum immunoglobulin equivalents (Table 3).

## DISCUSSION

Our study aimed to survey the presence of sIgA (mucosal IgA) in saliva referred to as salivary IgA, together with IgA and IgG antibodies in serum, capable of reacting with SARS-CoV-2 among Japanese people of a wide range of age from infants to the elderly who had been unexposed to the virus. The results evidenced the presence of saliva IgA antibodies reactive with the SARS-CoV-2 spike-1 antigen at detectable levels in a substantial fraction of enrolled individuals of all ages. Indeed, 95/180 (52.78%) of overall individuals were positive for anti-SARS-CoV-2 saliva IgA antibodies. In addition, we found that there may be a negative trend in positivity for the virus-reactive saliva IgA according to age; the highest and the lowest prevalence of positivity were observed for the youngest age group (<20 years, 65.85%) and the oldest age group (≥51 years, 40.38%), respectively, compared with two intermediate aged adult groups.

Recently, a huge body of evidence has accumulated that susceptibility to SARS-CoV-2 infection generally increases with age. Compared with younger/middle-aged adults, children are less susceptible to the virus infection, while the estimated susceptibility in older adults is considerably higher.[23–27] In addition, the low fragility of children and adolescents against SARS-CoV-2 has also become a matter of epidemiological and virological concern and intensive studies have been performed to identify the immune mechanisms implicated. As a result, several protective mechanisms including differences in the timing and nature of the induced cytokine responses and the high amounts of ACE2 expressed, as well as trained immunity acquired from frequent viral infections and/or routine vaccinations, have been hypothesized.[28] High positivity for SARS-CoV-2-reactive saliva IgA antibodies in minor participants, compared with adult participants, as observed in the present study may contribute to lowering their susceptibility to the virus. As far as adult individuals are concerned, our results are in accordance with the data presented previously by Tsukinoki and colleagues, who described a rate of positive SARS-CoV-2 cross-reactive saliva IgA antibodies as high as 46.7% among the virus-uninfected professional workers of Kanagawa Dental University Hospital, being significantly lower in the frequency of positivity for individuals aged ≥ 50 years compared with those aged ≤ 49 years.[22] The results of these two studies indicate that

**TABLE 2** Proportion positive for saliva IgA, serum IgA, and serum IgG antibodies reactive with SARS-CoV-2 in an adult participant group (age ≥ 20 years) of the study population stratified by gender and age subgroups

| Variable | Number of participants | Proportion of participants, % (95% CI)[a] | Positive for saliva IgA antibodies | | | Positive for serum IgA antibodies | | | Positive for serum IgG antibodies | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *N* | Proportion, % (95% CI)[a] | *P* value[b] | *N* | Proportion, % (95% CI)[a] | *P* value[b] | *N* | Proportion, % (95% CI)[a] | *P* value[b] |
| Overall | 139 | 100 | 68 | 48.92 (40.35–57.53) | | 24 | 17.27 (11.39–24.59) | | 17 | 12.23 (7.29–18.86) | |
| Gender | | | | | | | | | | | |
| Male | 71 | 51.08 (42.47–59.65) | 36 | 50.70 (38.56–62.78) | 0.735 | 13 | 18.31 (10.13–29.27) | 0.824 | 8 | 11.27 (4.99–21.00) | 0.799 |
| Female | 68 | 48.92 (40.35–57.53) | 32 | 47.06 (34.83–59.55) | | 11 | 16.18 (8.36–27.10) | | 9 | 13.24 (6.23–23.64) | |
| Age, years | | | | | | | | | | | |
| 20–35 | 45 | 32.37 (24.69–40.83) | 23 | 51.11 (35.77–66.30) | 0.259 | 10 | 22.22 (11.20–37.09) | 0.564 | 9 | 20.00 (9.58–34.60) | 0.055 |
| 36–50 | 42 | 30.22 (22.72–38.57) | 24 | 57.14 (40.96–72.28) | | 6 | 14.29 (5.43–28.54) | | 6 | 14.29 (5.43–28.54) | |
| ≥51 | 52 | 37.41 (29.36–46.01) | 21 | 40.38 (27.01–54.90) | | 8 | 15.38 (6.88–28.08) | | 2 | 3.85 (0.47–13.21) | |

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]Estimated using exact binominal distribution.
[b]Estimated by $\chi^2$ test.

**TABLE 3** Correlation of the positivity for SARS-CoV-2 of saliva IgA antibodies with equivalents of serum IgA and IgG antibodies in an adult participant group (aged 20 years; $n = 139$) of the study population

| Antibodies | Correlation ($r$)[a] | 95% CI[a] | *P* value[a] |
|---|---|---|---|
| Serum IgA | −0.049 | −0.214 to −0.118 | 0.57 |
| Serum IgG | 0.106 | −0.061 to −0.268 | 0.21 |

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]Calculated by Pearson's correlation analysis.

there is a decrease in SARS-CoV-2-reactive saliva IgA antibodies with increasing age and are in line with preceding research.[29–31]

There are numerous reported human studies which indicated the association of low levels of or decreases in saliva IgA antibodies with the incidence of upper respiratory infections, mostly the common cold, in various study cohorts, such as infants,[32] healthy children,[33,34] children with Down's syndrome,[35] elite athletes,[36,37] individuals during intense physical training,[38] and those who had undergone tonsillectomy or adenoidectomy in childhood.[39] These results suggest that individuals who are deficient in saliva IgA, particularly those who are negative for saliva IgA antibodies reactive with SARS-CoV-2, may be at higher risk for the virus infection. In this context, the results from the present study showing unexpectedly high rates of positivity for SARS-CoV-2-reactive saliva IgA antibodies seen in a study population of the virus-unexposed Japanese participants could be associated with the extremely low incidence of COVID-19 in Japan.

We also found that SARS-CoV-2-reactive serum IgA and/or IgG antibodies are detected from some areas of the participants. However, there was no correlation in the positivity for, or the level of, the virus-reactive serum IgA or IgG antibodies with equivalent saliva IgA antibodies. This may be probably due to different mechanisms involved in the regulation of the generation and/or the dynamics of each type of immunoglobulin. It is well accepted that sIgA, a major component of saliva IgA, is generated and released at mucosal inductive site tissues, while its serum counterpart is, like IgG, derived from a distinct source, the bone marrow.[40,41] Presumably, both IgA and IgG antibodies in serum may complement the role of sIgA antibodies in mucosal secretions. Although the actual nature of the SARS-CoV-2-reactive sIgA antibodies remains elusive, natural polyreactive sIgA ("natural" sIgA) antibodies, which are suggested to be able to naturally bind and neutralize multiple targets,[42,43] are the most probable. Future studies on this issue will be warranted.

The present study has both strengths and limitations. By implementing this serological survey in a cross-sectional study with a wide age range of healthy Japanese participants who had been unexposed to SARS-CoV-2, we were able to collect preliminary but unprecedented epidemiological data regarding the prevalence and levels of assumable "natural"

sIgA antibodies that exhibit reactivity with the virus. To the best of our knowledge, our survey, along with the preceding study by one of us,[22] is the only available study that provides data useful for considering a role of such innate sIgA antibodies as a possible barrier to mucosal infections including SARS-CoV-2 infection.

Our study has several limitations. First, our sample size was not large, limiting the robustness of our findings in saliva, as well as in serum. If such samples could become increasingly available, statistical power for the analyses presented here will be increased. Secondly, we do not know whether and to what level of the sIgA antibodies in saliva and/or other mucosal secretions, are protective through neutralization against SARS-CoV-2 infection. Thirdly, because of the lack of follow-up data, it was also not possible to directly correlate negativity for or low levels of SARS-CoV-2-reactive saliva IgA antibodies with the feasibility to have the virus infection. Nevertheless, the results obtained from the present study still lead us to favor the hypothesis that "natural" sIgA antibodies could contribute to lower the susceptibility to SARS-CoV-2 infection and to play a positive role in achieving herd immunity against COVID-19. Fourthly, the possibility that a certain number of participants had had asymptomatic COVID-19 in the past could not be ruled out. Because we were unable to distinguish "natural" sIgA antibodies reactive with SARS-CoV-2 from the virus infection-induced specific sIgA antibodies. Fifthly, we are uncertain as to how the underlying mechanisms involved in the production of such assumable "natural" sIgA antibodies are different between the Japanese people and other nations because, to our knowledge, there is no published study dealing with this important open question yet to be clarified. Relevantly, it has been shown that selective IgA deficiency (sIgAD), defined as an isolated deficiency of IgA in the blood and secretions, is found at the lowest frequency of 1 in 18,550 in Japanese and a high of 1:142 among Caucasians,[44] and that such a low frequency of sIgAD positively correlates with the lower prevalence of COVID-19 in Japan in comparison with other countries.[45,46] These findings can be considered to support our postulation that we Japanese people might inherently possess a higher ability to produce IgA antibodies, including a repertoire of those reactive with working on SARS-CoV-2, than other nations.

In conclusion, our preliminary results, obtained with SARS-CoV-2-unexposed Japanese participants, showed the presence of assumable "natural" sIgA antibodies reactive with the virus at a considerably high rate. This could be related with a relatively low incidence of COVID-19 in Japan.

## AUTHOR CONTRIBUTIONS

*Conceptualization*: Keiichi Tsukinoki, Tetsuro Yamamoto. *Methodology*: Keiichi Tsukinoki. *Investigation*: Wakako Sakaguchi. *Sample collection*: Jiro Saito, Keiichiro Iguchi, Yoshinori Inoue, Shigeru Ishii, Chikatoshi Sato, Mina Yokoyama, Yuki Shiraishi, Noriaki Kato, Akio Makabe, Akihiro Saito. *Funding acquisition*: Tetsuro Yamamoto. *Analysis of data*: Hiroyasu Shimada. *Project administration*: Keiichi Tsukinoki, Tetsuro Yamamoto. *Supervision*: Masanori Tanji, Isao Nagaoka, Juri Saruta, Tetsutaro Yamaguchi, Shigenari Kimoto. *Writing*: Hideyo Yamaguchi.

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## CONFLICTS OF INTEREST

As employees of EPS, the following people are paid by EPS: Tetsuro Yamamoto, Yuki Shiraishi, Noriaki Kato, Hiroyasu Shimada, Akio Makabe, Akihiro Saito, Masanori Tanji, and Hideyo Yamaguchi. The remaining authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All data associated with this study are present in the paper.

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