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SHORT COMMUNICATION

Tear antibodies to SARS-CoV-2: implications for transmission

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Abstract

Objectives. SARS-CoV-2 can be transmitted by aerosols, and the ocular surface may be an important route of transmission. Little is known about protective antibody responses to SARS-CoV-2 in tears after infection or vaccination. We analysed the SARS-CoV-2-specific IgG and IgA responses in human tears after either COVID-19 infection or vaccination. **Methods.** We measured the antibody responses in 16 subjects with COVID-19 infection for an average of 7 months before, and 15 subjects before and 2 weeks post-Comirnaty (Pfizer-BioNtech) vaccination. Plasma, saliva and basal tears were collected. Eleven pre-pandemic individuals were included as healthy controls. **Results.** IgG antibodies to spike and nucleoprotein were detected in tears, saliva and plasma from subjects with prior SARS-CoV-2 infection in comparison with uninfected controls. While receptor-binding domain (RBD)-specific antibodies were detected in plasma, minimal RBD-specific antibodies were detected in tears and saliva. By contrast, high levels of IgG antibodies to spike and RBD, but not nucleoprotein, were induced in tears, saliva and plasma of subjects receiving 2 doses of the Comirnaty vaccine. Increased levels of IgA1 and IgA2 antibodies to SARS-CoV-2 antigens were detected in plasma following infection or vaccination but were unchanged in tears and saliva. Comirnaty vaccination induced high neutralising Abs in the plasma, but limited neutralising antibodies were detected in saliva or tears. **Conclusion.** Both infection and vaccination induce SARS-CoV-2-specific IgG antibodies in tears. RBD-specific IgG antibodies in tears were induced by vaccination but were not present 7 months post-infection. This suggests the neutralising antibodies may be low in the tears late following infection.

Keywords: antibodies, COVID-19, neutralisation, saliva, SARS-CoV-2, tears

INTRODUCTION

SARS-CoV-2, the cause of the COVID-19 pandemic, is commonly acquired via airborne transmission. Observational studies show that the wearing of spectacles is associated with reduced SARS-CoV-2 acquisition, suggesting SARS-CoV-2 may be acquired through the ocular surface.[1,2] Similarly, eye protection initiatives have been associated with reduced health care-associated outbreaks of COVID-19.[3] As a result of these findings, eye protection is widely recommended in healthcare settings.

Despite these observations and recommendations, relatively little is known about immunity to SARS-CoV-2 at the ocular surface, even though this may be an important route of transmission. Recent studies have documented SARS-CoV-2-specific antibody responses in tears following SARS-CoV-2 infection,[4,5] although the specificity, durability and functional significance of the antibodies detected after infection remain unclear.

Vaccination is highly protective from SARS-COV-2 infection, and considerable evidence points towards neutralising antibodies as a key correlate of protection.[6] Most neutralising antibodies target the RBD of the spike protein that is critical in binding to cellular receptors.[7] RBD-specific antibodies correlate strongly with functional neutralising antibody assays.[8] Little is known about whether vaccination against SARS-CoV-2 via the intramuscular route induces SARS-COV-2 antibodies in tears, especially RBD-specific antibodies. Herein, we collected basal tears, saliva and plasma samples from subjects with prior COVID-19 infection or vaccination and analysed IgG and IgA antibody responses to a range of SARS-CoV-2 antigens.

RESULTS

In all, 42 participants were enrolled: 11 healthy controls, 16 with prior SARS-CoV-2 infection with a mean of 210 days from previous symptom onset and 15 Comirnaty vaccine recipients, where samples were taken prior to vaccination and a mean of 13 days following their second vaccine dose (Table 1).

IgG antibodies to spike proteins ST, S1 and S2 and to the nucleoprotein (NP) protein were detected in tears, saliva and plasma from subjects with prior SARS-CoV-2 infection in comparison with uninfected controls. While RBD-specific antibodies were detected in convalescent plasma, RBD-specific antibodies were not detectable in convalescent tears and saliva in comparison with healthy controls (Figure 1a).

The Comirnaty vaccine is highly protective for SARS-CoV-2 infection.[9] We found vaccination induced high levels of spike-specific IgG antibodies (including RBD) in tears, saliva and plasma (Figure 1b). As expected, IgG responses to NP were minimal, since the Comirnaty vaccine does not express this antigen.

IgA antibodies at mucosal surfaces are important in protection from multiple infectious diseases,[10] and we previously observed IgA1 and IgA2 antibodies to spike in the plasma of subjects for up to 4 months after SARS-CoV-2 infection.[11] We studied IgA1 and IgA2 antibodies in the plasma, tears and saliva samples of subjects at a mean of 7 months after SARS-CoV-2 infection (Figure 2a and b). Plasma IgA1 and IgA2 responses were detected to most spike SARS-CoV-2 antigens in plasma but uniformly at lower levels compared to IgG antibodies. Plasma IgA1 and IgA2 responses were not detectable to NP 7 months following infection. No IgA1 and IgA2 responses to SARS-CoV-2 antigens were detected in tears and saliva. Background levels of cross-reactive IgA1 and IgA2 antibodies in uninfected controls were uniformly higher than in IgG responses.

Intramuscular vaccination is not considered an optimal vaccination route to induce IgA responses at mucosal surfaces, although some IgA responses can be induced by this vaccination method.[12,13] Plasma IgA1 and IgA2 responses were detected to all five SARS-CoV-2 antigens early after Comirnaty vaccination but uniformly at lower levels than IgG antibodies (Figure 2c and d). Although non-significant increases of IgA1 and IgA2 responses to spike trimer were detected in saliva (fold change 2.5 and 2.7, respectively) and tears (fold change 1.9 and 3.1, respectively) after vaccination, overall IgA responses to spike subunit proteins in the mucosal samples were not detectable. Background pre-vaccination levels of IgA1 and IgA2 antibodies

Table 1. Demographics and clinical characteristics of healthy individuals, COVID-19 patients and Comirnaty (Pfizer-BioNtech) vaccine recipients

Variables	Healthy controls (n = 11)	COVID-19 patients (n = 16)	Vaccine recipients (n = 15)
Age, mean (range), years	46.0 (21–64)	52.7 (22–76)	34.0 (25–57)
Gender			
Female	3 (27.3%)	8 (50.0%)	10 (66.7%)
Male	8 (72.7%)	8 (50.0%)	5 (33.3%)
Time from symptom onset till sample collection, mean (range), days		210.4 (65–249)	
Severity			
Mild		6 (37.5%)	
Moderate		8 (50.5%)	
Severe		2 (12.5%)	
Received Comirnaty (Pfizer-BioNtech) vaccine			15 (100.0%)
Time after 1st vaccine to sample collection, mean (range), days			37.3 (33–47)
Time after 2nd vaccine to sample collection, mean (range), days			13.4 (12–18)

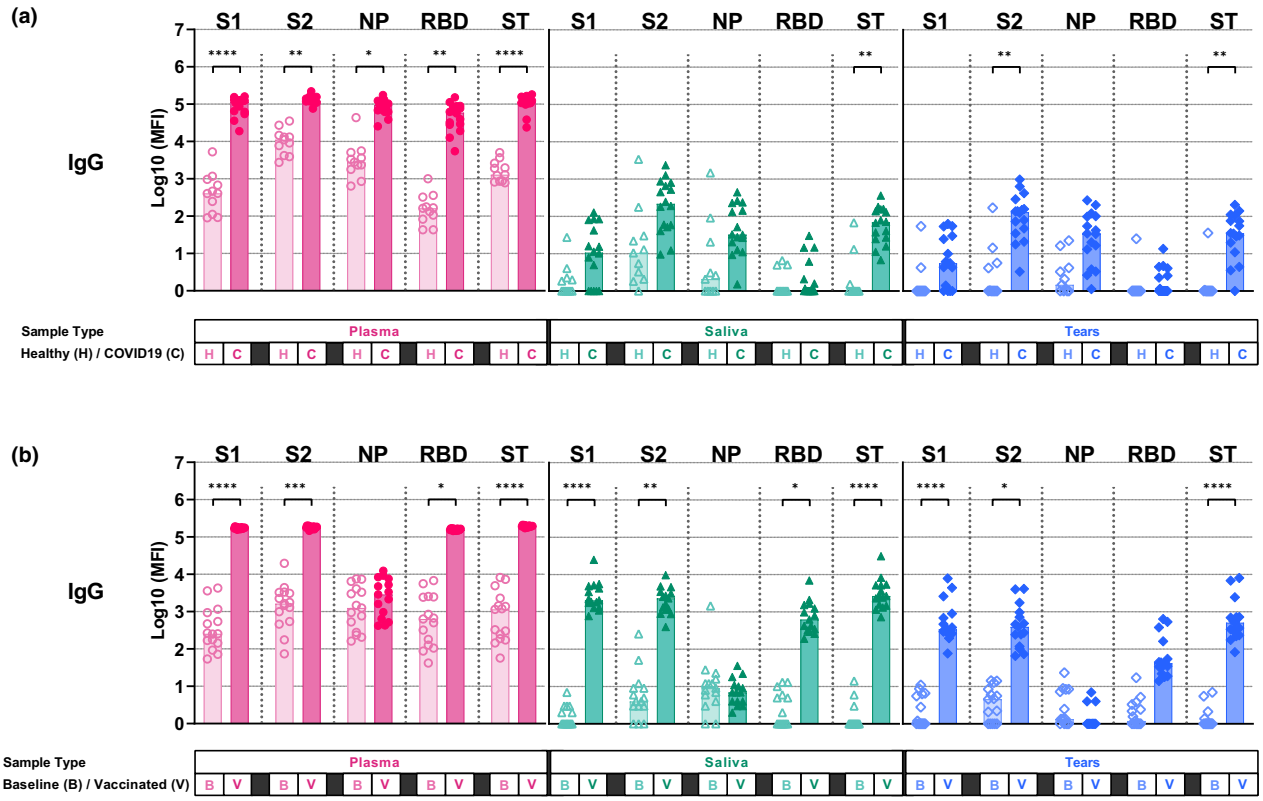

Figure 1. Anti-SARS-CoV-2 IgG found in plasma, saliva and tears of **(a)** convalescent COVID-19 patients and **(b)** Comirnaty (Pfizer-BioNTech) vaccinees. The presence of IgG specific for SARS-CoV-2 spike 1 (S1), spike 2 (S2), nucleoprotein (NP), receptor-binding domain (RBD) and whole spike trimer (ST) was compared in samples from **(a)** both healthy individuals (H, n = 11) and convalescent COVID-19 patients (C, n = 16; Kruskal–Wallis test), **(b)** as well as in paired baseline pre-vaccination (B) and 2 weeks post-second dose vaccinated (V) samples from Comirnaty (Pfizer-BioNTech) vaccinees (n = 15, Friedman test). MFI readings from tears and saliva samples were normalised to a final dilution of 1:200. *P*-values*:* * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

were again uniformly higher than those of IgG responses.

To evaluate whether mucosal antibodies elicited by intramuscular vaccination could induce sufficient neutralising activity for mucosal protection, we assessed paired plasma, saliva and tear samples (baseline and vaccinated) with sufficient sample volumes with our published

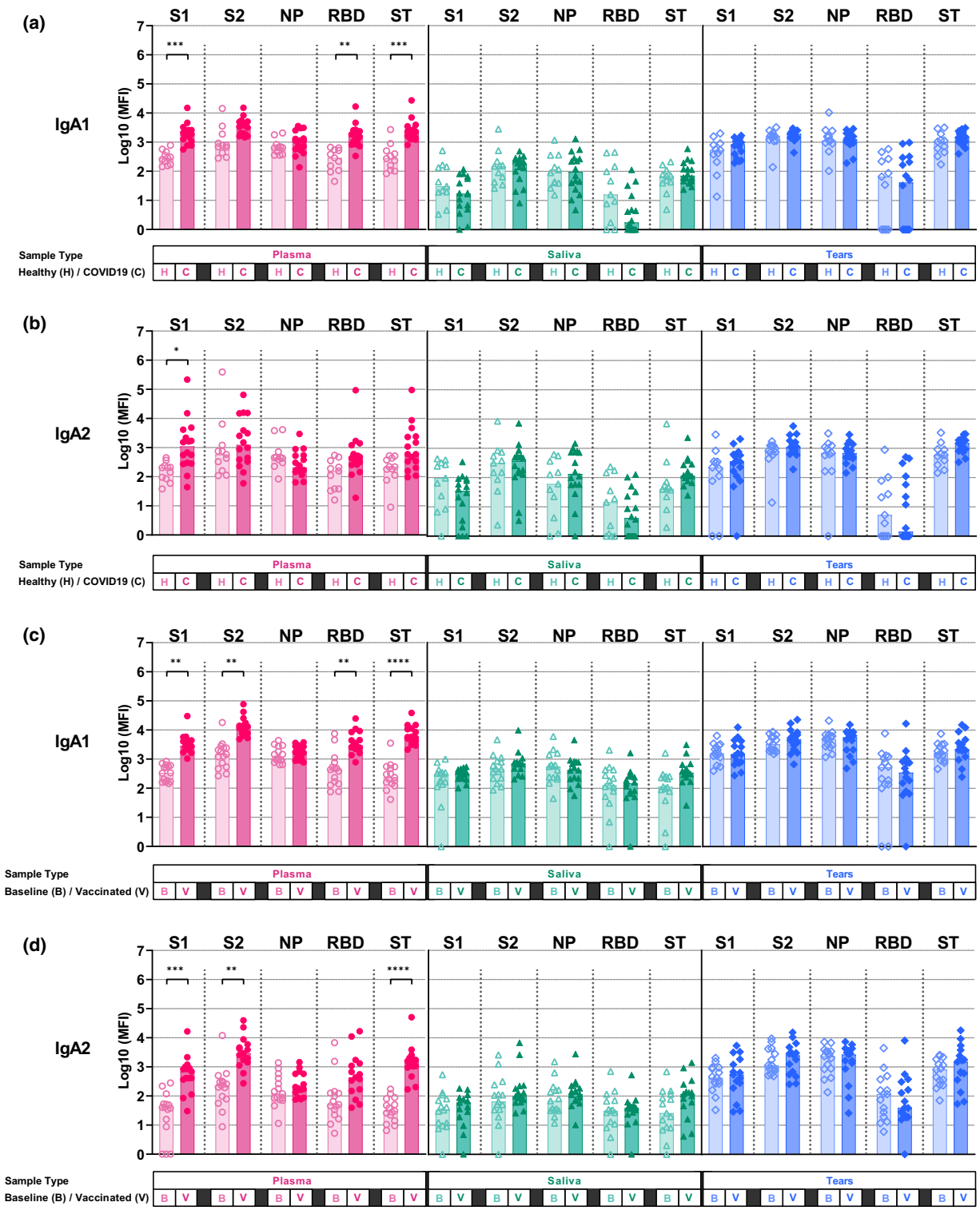

Figure 2. Anti-SARS-CoV-2 IgA1 and IgA2 found in plasma, saliva and tears of **(a, b)** convalescent COVID-19 patients and **(c, d)** Comirnaty (Pfizer-BioNTech) vaccinees. The presence of IgA1 and IgA2 specific for SARS-CoV-2 spike 1 (S1), spike 2 (S2), nucleoprotein (NP), receptor-binding domain (RBD) and whole spike trimer (ST) was compared in samples from **(a, b)** both healthy individuals (H, $n = 11$) and convalescent COVID-19 patients (C, $n = 16$; Kruskal–Wallis test), **(c, d)** as well as in paired baseline pre-vaccination (B) and 2 weeks post-second dose vaccinated (V) samples from Comirnaty (Pfizer-BioNTech) vaccinees ($n = 15$. Friedman test). MFI readings from tears and saliva samples were normalised to a final dilution of 1:200. *P*-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

SARS-CoV-2 inhibition multiplex assay.[14] Here, we measured the ability of the samples to inhibit the binding of SARS-CoV-2 spike 1 to ACE2. While we detected a sharp increase in neutralising activity in plasma after vaccination, neutralising activity was only marginally increased in saliva, and no differences were observed in tears after vaccination (Figure 3ai-vi).

Furthermore, to verify our observations, we also performed a cell-based neutralisation assay[14] using Vero cells infected with SARS-CoV-2 in the presence of paired plasma and saliva samples

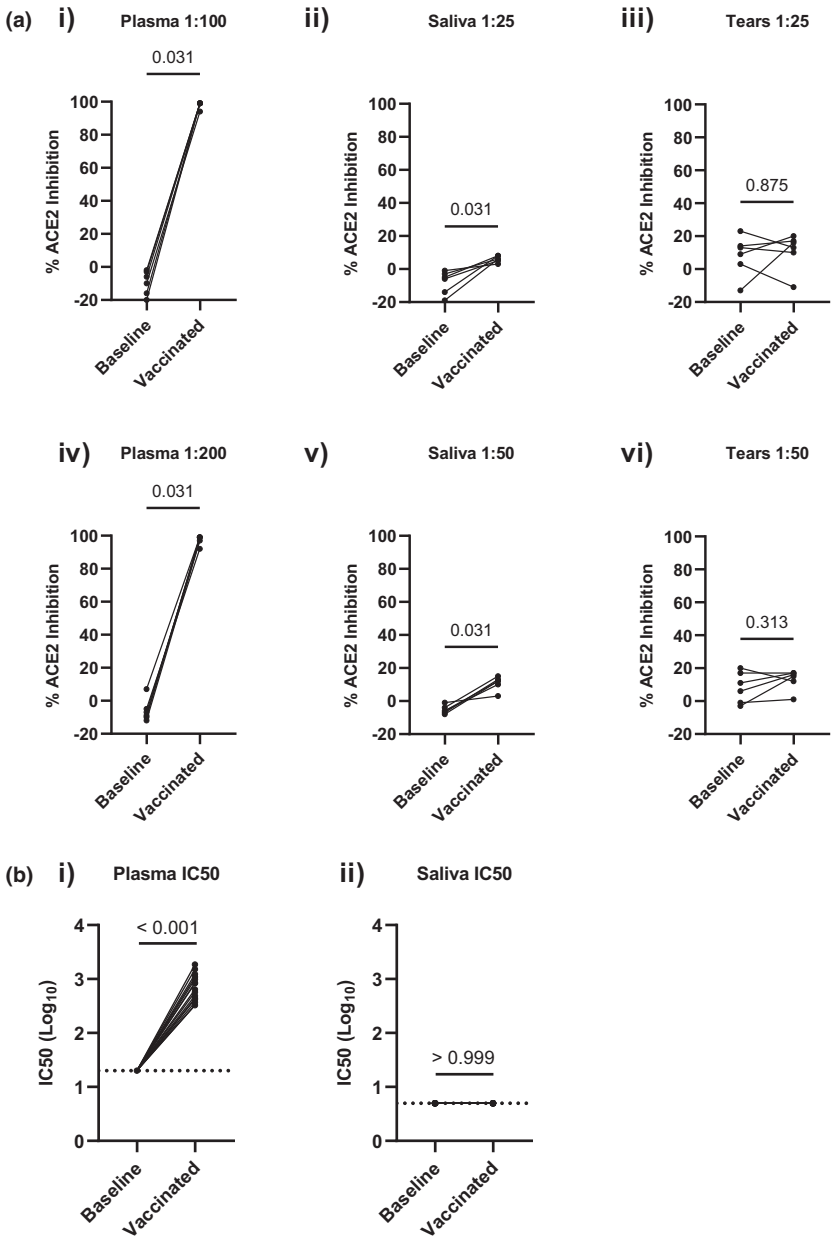

Figure 3. Neutralising activity in plasma, saliva and tears of Comirnaty (Pfizer-BioNTech) vaccinees. **(a)** Neutralising activity in paired pre-vaccination and vaccinated plasma **(i, iv)**, saliva **(ii, v)** and tear samples **(iii, vi)** from Comirnaty (Pfizer-BioNTech) vaccinees (n = 6) was assessed with the competitive S1-ACE2 bead-based inhibition assay (Wilcoxon test) over 2 dilutions. **(b)** Neutralising activity in paired serially diluted (7 × 2.5-fold dilutions) pre-vaccination and vaccinated plasma **(i)** (1:20–1:4882.8 final dilution; n = 15) and saliva samples **(ii)** (1:5–1:1220.7 final dilution; n = 15) from Comirnaty (Pfizer-BioNTech) vaccinees was assessed with a cell-based microneutralisation assay (Wilcoxon test). IC50 values were determined using 4-parameter nonlinear regression.

($n = 6$). Tear samples were unable to be assessed using this cell-based assay because of insufficient sample volumes. We found that vaccinated plasma but not saliva samples effectively neutralised SARS-CoV-2 infection (Figure 3bi and ii). To investigate whether the enzymes in saliva were interfering with neutralising antibodies, we spiked baseline saliva samples with recombinant neutralising monoclonal antibodies and found that the monoclonal antibodies in spiked saliva samples still potently neutralise SARS-CoV-2 infection (Supplementary figure 2).

DISCUSSION

Previous work has identified SARS-CoV-2-specific antibodies in the tears of subjects following COVID-19, although little is known about the specificity or longevity of tear antibodies,[4] and nothing has been reported on tear antibodies following COVID-19 vaccination to our knowledge. We found that although SARS-CoV-2 antibodies are detectable in the tears and saliva of subjects with prior SARS-CoV-2 infection, levels of antibodies to RBD were low or undetectable by 7 months following infection. Since most neutralising antibodies bind to the RBD portion of spike, this suggests that the neutralising antibodies in these mucosal samples are low. Additional studies of the direct virus neutralisation capacity of tear antibodies will be helpful, although most assays require larger sample volumes that are difficult to obtain with tears. Re-infection with SARS-CoV-2 is being increasingly reported, and we speculate that low mucosal antibodies may leave subjects with prior SARS-CoV-2 infection vulnerable to re-infection through these sites, particularly through eye surfaces.

Importantly, this report demonstrated vaccination induced high levels of tear and saliva IgG antibodies, including RBD. This suggests that vaccination, at least early after the course is completed, induces key antibodies at the ocular surface that may help protect from the acquisition of SARS-CoV-2. While we were unable to detect neutralising protection in post-vaccinated tear and only limited neutralising antibodies in saliva samples, it is feasible that the SARS-CoV-2 IgG present could still induce non-neutralising protection, warranting future investigation. The relative levels and durability of tear antibodies induced by differing COVID-19 vaccines also remain to be determined. This may be an important factor governing the waning of vaccine-induced immunity and the need for future booster vaccinations.

We found that SARS-CoV-2-specific IgA responses in tears and saliva were low or non-detectable either 7 months following infection or early following vaccination. As a mucosal infection, one might expect IgA responses in tears and saliva following infection. The lack of IgA in tears and saliva following infection may reflect the waning of immunity. We have previously reported IgA responses wane rapidly in plasma post-infection.[11] Similarly, strong plasma IgA responses were induced after a single vaccine dose of phase clinical trials I of a protein-MF59-adjuvanted spike glycoprotein clamp but rapidly waned despite a second dose.[15] However, we do acknowledge that our study is limited by lack of access to tear and saliva samples collected during acute SARS-CoV-2 infection. Further studies of IgA responses in tears earlier following infection are warranted.

The lack of IgA responses in tears and saliva following intramuscular Comirnaty vaccination may reflect that this route is relatively poor at inducing mucosal IgA responses.[12] Although this vaccine is highly protective, whether modified regimens that induce IgA responses at mucosal surfaces are more durably protective warrants further study. Further study of secretory IgA, more specific to mucosal IgA, is also warranted. In general, we also noted that background IgA1 and IgA2 responses to SARS-CoV-2 antigens were high in the tears and saliva of uninfected and unvaccinated subjects. It is possible that prior exposure to other human coronaviruses may induce high levels of cross-reactive IgA antibodies at baseline, and studies of antibodies to other coronaviruses are warranted. More refined methods to detect SARS-CoV-2 IgA antibodies in tears and saliva are needed.

In conclusion, COVID-19 infection and Comirnaty vaccination induced SARS-CoV-2-specific IgG antibodies in tears and saliva. These non-neutralising IgG antibodies in tears and saliva following vaccination may still have play protective roles at the mucosa and warrrant further investigation. Our findings reinforce the need for widespread vaccination and eye protection in settings where this is not yet possible.

METHODS

We enrolled participants (1) with and without prior SARS-CoV-2 infection from a previously described cohort[16] and (2) prior to and following the Comirnaty (Pfizer-BioNTech; Brookyln, NY, USA; Mainz, Germany) vaccine to donate blood, saliva and tears (Table 1). Basal (non-stimulated) tear samples ($\sim$7 μL per eye) were collected by capillary flow (Drummond Scientific, Broomall, PA, USA) from the inferior tear meniscus as previously reported.[17] Saliva was collected by SalivaBio Oral Swabs (Salimetrics, Carlsbad, CA, USA) following manufacturer's instructions.

SARS-CoV-2-specific IgG, IgA1 and IgA2 antibodies in plasma (1:200), saliva (1:5 and 1:50) and tears (1:5 and 1:50) from the respective cohorts (1 and 2) were assessed by a customised multiplex bead array consisting of five SARS-CoV-2 proteins, including a whole spike trimer (ST), the spike 1 (S1; Sino Biological, Beijing, China), spike 2 (S2; Acro Biosystems, Newark, NJ, USA), RBD of spike and NP (Acro Biosystems) as previously described.[18] The SIVgp120 protein (Sino Biological) and uncoupled BSA-blocked beads were included as negative controls for background subtraction.[18] Sample concentrations used in the array were chosen based off a dilution series (examples shown in Supplementary figure 1). In short, antigen-coupled beads were incubated with the respective samples on a shaker overnight at 4°C, before being washed and incubated with PE-conjugated detection antibodies (Pan IgG, IgA1, IgA2; SouthernBiotech, Birmingham, AL, USA) for 2 h with shaking at room temperature.[18] Beads were washed again and read on the Flexmap3D.

To assess the neutralising activity of post-vaccinated plasma, saliva and tears using minimal sample volume, we used an in-house competitive S1-ACE2 bead-based inhibition assay as previously published.[14] Briefly, S1-coupled beads were incubated with avi-tagged biotinylated ACE2 in the presence of the respective diluted samples in 5% Triton X-100 for 2 h with shaking at room temperature. After washing, the beads were incubated in diluted streptavidin R-phycoerythrin conjugate (SAPE; Thermo Fisher Scientific, Scoresby, Australia) for 1 h with shaking at room temperature. Diluted R-phycoerythrin biotin-XX conjugate (Thermo Fisher Scientific) was then added to the beads and incubated for another hour with shaking at room temperature. Beads were washed and read on the Flexmap3D.

To further assess the neutralising activity of post-vaccinated plasma and saliva samples, we used a cell-based virus microneutralisation assay with ELISA-based readout as previously described.[14] In short, SARS-CoV-2 isolate CoV/Australia/VIC01/2020 passaged in Vero cells was incubated at 2000 $TCID_{50}$ mL^{-1} with respective serially diluted samples for 1 h at 37°C. The sample virus mixtures were then added to plates seeded with Vero cells and incubated for 48 h at 37°C. After 48 h, the cells are fixed, washed, permeabilised and blocked, before being incubated with diluted rabbit polyclonal anti-SARS-CoV N antibody (Rockland, Limerick, ME, USA) for 1 h at room temperature. After washing, the plates were incubated with diluted goat anti-rabbit IgG-HRP (Abcam, Cambridge, UK) for another hour at room temperature. Finally, the plates were washed, developed with TMB (Sigma-Aldrich, St. Louis, MO, USA) and stopped with 0.15 M H_2SO_4, before being read on a plate reader at 450 nm. OD values were then used to calculate percentage neutralisation with the following formula: ('virus + cells' − 'sample') ÷ ('virus + cells' − 'cells only') × 100. IC50 values were determined using 4-parameter nonlinear regression in GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) with curve fits constrained to have a minimum of 0% and maximum of 100% neutralisation.

All participants provided written informed consent; the study was approved by the University of Melbourne human research and ethics committee (2056689 and 21198153983). Statistical analysis was performed with GraphPad Prism 9 (GraphPad Software). Antibody levels between antigens (ST, S1, S2, RBD and NP) within each sample type (plasma, tears and saliva) were compared using Kruskal–Wallis test (1) or Friedman test (2), respectively, followed by Dunn's test for multiple comparisons.

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AUTHOR CONTRIBUTIONS

Kevin J Selva: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-review & editing. **Samantha K Davis:** Investigation; Methodology; Writing-review & editing. **Ebene R Haycroft:** Investigation; Methodology; Writing-review & editing. **Wen Shi Lee:** Investigation; Methodology; Writing-review & editing. **Ester Lopez:** Investigation; Methodology. **Arnold Reynaldi:** Formal analysis. **Miles P Davenport:** Formal analysis. **Helen E Kent:** Investigation. **Jennifer A Juno:** Investigation. **Amy W Chung:** Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing-review & editing. **Stephen J Kent:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing-original draft.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.