BRIEF COMMUNICATION

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Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection

Salma Sheikh-Mohamed[1], Baweleta Isho[1,17], Gary Y. C. Chao[1,17], Michelle Zuo[1,17], Carmit Cohen[2], Yaniv Lustig[2,3,4], George R. Nahass[5,6], Rachel E. Salomon-Shulman[5], Grace Blacker[5], Mahya Fazel-Zarandi[7], Bhavisha Rathod[7], Karen Colwill[7], Alainna Jamal[7], Zhijie Li[8], Keelia Quinn de Launay[7,9], Alyson Takaoka[7,9], Julia Garnham-Takaoka[7,9], Anjali Patel[7,9], Christine Fahim[9], Aimee Paterson[7], Angel Xinliu Li[7], Nazrana Haq[7], Shiva Barati[7], Lois Gilbert[7], Karen Green[7], Mohammad Mozafarihashjin[7], Philip Samaan[10], Patrick Budylowski[10], Walter L. Siqueira[11], Samira Mubareka[10,12], Mario Ostrowski[1,13,14], James M. Rini[8,15], Olga L. Rojas[16], Irving L. Weissman[5], Michal Caspi Tal[5], Allison McGeer[7], Gili Regev-Yochay[2], Sharon Straus[9]✉, Anne-Claude Gingras[7,8]✉ and Jennifer L. Gommerman[1]✉

Although SARS-CoV-2 infects the upper respiratory tract, we know little about the amount, type, and kinetics of antibodies (Ab) generated in the oral cavity in response to COVID-19 vaccination. We collected serum and saliva samples from participants receiving two doses of mRNA COVID-19 vaccines and measured the level of anti-SARS-CoV-2 Ab. We detected anti-Spike and anti-Receptor Binding Domain (RBD) IgG and IgA, as well as anti-Spike/RBD associated secretory component in the saliva of most participants after dose 1. Administration of a second dose of mRNA boosted the IgG but not the IgA response, with only 30% of participants remaining positive for IgA at this timepoint. At 6 months post-dose 2, these participants exhibited diminished anti-Spike/RBD IgG levels, although secretory component-associated anti-Spike Ab were more stable. Examining two prospective cohorts we found that participants who experienced breakthrough infections with SARS-CoV-2 variants had lower levels of vaccine-induced serum anti-Spike/RBD IgA at 2–4 weeks post-dose 2 compared to participants who did not experience an infection, whereas IgG levels were comparable between groups. These data suggest that COVID-19 vaccines that elicit a durable IgA response may have utility in preventing infection.

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INTRODUCTION

SARS-CoV-2 is a novel and highly contagious respiratory virus that has quickly spread across the globe. The virus uses a protein called Spike and its associated receptor binding domain (RBD) to interact with angiotensin converting enzyme 2 (ACE2) on host cells[1]. Interaction between viral Spike/RBD and ACE2 on the cell surface is the first essential step in SARS-CoV-2 infection, and expression of ACE2 on epithelial cells of the upper respiratory tract (URT) renders them susceptible to aerosolized virus. Thus, immunity in the oral and nasal mucosa is an important first line of defense against the development of COVID-19[2].

Saliva is an important biofluid that can provide information about the mucosal antibody (Ab) response to SARS-CoV-2[3]. Indeed, salivary gland epithelial cells express ACE2 and harbor a significant population of IgA-producing plasma cells[4]. Secretory IgA (SIgA) in the saliva exists as IgA dimers that are associated with the secretory component, a proteolytic cleavage product which remains bound to IgA after it is transported across epithelial cells via the polymeric Ig receptor (pIgR)[5]. Secretory polymeric IgA has been shown to have potent neutralizing activity against SARS-CoV-2 in vitro[6].

We and others have shown that IgM, IgG and IgA Ab against the SARS-CoV-2 Spike and RBD proteins are readily detected in the

[1]Department of Immunology, University of Toronto, Toronto, ON, Canada. [2]Sheba Medical Center Tel Hashomer, Ramat Gan, Sackler School of Medicine, Tel Aviv University, Tel Aviv-Yafo, Israel. [3]Central Virology Laboratory, Public Health Services, Ministry of Health, Sheba Medical Center, Tel-Hashomer, Tel-Aviv University, Tel Aviv-Yafo, Israel. [4]Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv-Yafo, Israel. [5]Institute for Stem Cell Biology and Regenerative Medicine and the Ludwig Cancer Center, Stanford University School of Medicine, Stanford, CA, USA. [6]University of Illinois-Chicago, College of Medicine, Chicago, USA. [7]Lunenfeld-Tanenbaum Research Institute, Sinai Health, Toronto, ON, Canada. [8]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada. [9]Li Ka Shing Knowledge Institute of St. Michael's Hospital, Unity Health Toronto, Toronto, ON, Canada. [10]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada. [11]College of Dentistry, University of Saskatchewan, Saskatoon, SK, Canada. [12]Sunnybrook Health Sciences Centre, Toronto, ON, Canada. [13]Department of Medicine, University of Toronto, Toronto, ON, Canada. [14]Keenan Research Centre for Biomedical Science, Toronto, ON, Canada. [15]Department of Biochemistry, University of Toronto, Toronto, ON, Canada. [16]Krembil Research Institute, University Health Network, Toronto, ON, Canada. [17]These authors contributed equally: Baweleta Isho, Gary Y. C. Chao, Michelle Zuo. ✉email: sharon.Straus@unityhealth.to; gingras@lunenfeld.ca; jen.gommerman@utoronto.ca

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saliva of COVID-19 acute and convalescent patients[3,7]. Whether COVID-19 vaccines delivered through the parenteral intramuscular route (i.m.) generate a similar salivary antibody response is unclear, and the nature and kinetics of this response are ill-characterized. Given the importance of mucosal immunity as a first line defense against SARS-CoV-2 infection we measured Spike/RBD-specific Ab in saliva samples from participants who had received either BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) vaccinations. We also determined whether levels of vaccine-induced anti-Spike/RBD IgG or IgA differed in people who subsequently experienced a SARS-CoV-2 infection. Collectively, our data show that a SIgA response is induced in ~30% of participants who received 2 doses of a SARS-CoV-2 mRNA vaccine, and that IgA may play an important role in protection against infection.

RESULTS

Detection of anti-Spike and anti-RBD antibodies in saliva from participants receiving COVID-19 mRNA vaccines

We first compared saliva from long-term care home (LTCH) workers that received either BNT162b2 or mRNA-1273 (Supplementary Fig. 1 and Supplementary Table 1) with pooled negative control saliva used to establish a cutoff (Supplementary Table 2a), and saliva from COVID-19 acute and convalescent patients as positive controls (Supplementary Table 2b). We expressed the data as a percentage relative to a pooled sample of saliva from COVID-19 acute and convalescent patients—the same pooled sample was present in each plate. We previously found that this method provided excellent plate-to-plate consistency and produced similar results as what we found when we normalized to total IgG/IgA[3]. Only 11% and 22% of vaccinated participants had a detectable IgM response to Spike and RBD respectively (data not shown). Focusing therefore on IgG and IgA responses, after two doses of mRNA vaccine 94% and 41% of participants were positive for anti-Spike IgG and IgA, and 93% and 20% of participants were positive for anti-RBD IgG and IgA Ab. While levels of anti-Spike/RBD IgG were similar to or exceeded that of COVID-19 convalescent patients (Fig. 1A, B), IgA levels were significantly lower (Fig. 1C, D). Furthermore, as we observed before in COVID-19 recovered patients[3], levels of salivary anti-Spike/RBD Ab positively correlated with anti-Spike/RBD Ab in the serum (Supplementary Fig. 2). In multivariable analysis, age and prior SARS-CoV-2 infection were independently associated with the salivary anti-Spike IgA response (Supplementary Table 3a). In contrast, male sex had a negative independent association with the salivary anti-Spike IgG response (Supplementary Table 3b) as has been observed before for COVID-19 and other vaccines[8,9]. Lastly, prior SARS-CoV-2 infection and time since vaccination were independently associated with higher and lower serum anti-RBD IgA levels, respectively (Supplementary Table 3c).

Longitudinal assessment of anti-Spike and anti-RBD antibodies in saliva from participants receiving 2 doses of COVID-19 mRNA vaccines at a 3 month dose interval

As of June 2021, although most LTCH workers had been fully vaccinated, significant sectors of the Canadian population had only been administered a single dose of a COVID-19 vaccine because the interval between dose 1 vs. dose 2 was extended as a dose sparing measure. Thus, we wished to ascertain if salivary Ab could be detected after a single dose of a COVID-19 mRNA vaccine, and how long these Ab would persist. We therefore collected samples from a second cohort of healthy adults that were followed longitudinally (Medical Sciences Building cohort—MSB; Supplementary Fig. 1 and Supplementary Table 4). These participants received 1 dose of BNT1162b2 and a second dose of BNT1162b2 3 months later, with samples taken at baseline, 2 weeks post-dose 1, 3 months post-dose 1, and 2 weeks post-dose 2. We observed that 97% and 93% of participants were positive for anti-Spike IgG and IgA, and 52% and 41% were positive for anti-RBD IgG and IgA Ab in their saliva 2 weeks post-dose 1 (Fig. 1E–H). Of note, 3 months after dose 1, the median level of salivary anti-Spike/RBD Ab had diminished nearly to baseline. Following administration of a second dose of mRNA, while antigen-specific IgG levels recovered upon administration of dose 2 as expected (Fig. 1E, F), a second dose did not further augment antigen-specific salivary IgA levels in most subjects, and only approximately 30% of participants remained positive for IgA after dose 2 (Fig. 1G, H). Therefore, the IgG and IgA response to COVID-19 mRNA vaccination differs upon administration of dose 2.

Detection of secretory component associated with anti-Spike and anti-RBD antibodies in saliva from participants receiving COVID-19 mRNA vaccines

The i.m. route induces an immune response in the axillary draining lymph node that is biased towards class switch to IgG rather than IgA. Thus, we were curious as to why we were able to detect an IgA anti-Spike/RBD response in the saliva, particularly after one dose of mRNA vaccine. We hypothesized that some IgA may be produced locally. To test this, we designed an ELISA to detect any secretory component associated with anti-Spike/RBD Ab. We determined that the secretory component signal associated with anti-SARS-CoV-2 salivary Ab could be out-competed with recombinant secretory component, and no anti-Spike/RBD secretory component signal was detected in pre-pandemic colostrum, demonstrating assay specificity (Supplementary Fig. 3). We then measured secretory component associated anti-SARS-CoV-2 Ab in the saliva of vaccinated LTCH participants who had received two doses of either BNT162b2 or mRNA-1273. We found that secretory component associated anti-Spike and anti-RBD Ab could be detected in 30% and 58% of participants, respectively, although the levels were significantly lower than what was observed in COVID-19 patients (Fig. 2A, B). The anti-SARS-CoV-2 associated secretory component signal was independent of prior SARS-CoV-2 exposure as we observed no significant difference in this signal comparing participants who were positive vs. negative for serum anti-nucleocapsid protein Ab (Fig. 2C, D). Of note, if we divided the LTCH cohort into those participants who were positive versus negative for anti-Spike/RBD IgA, we observed that the secretory component signal was only detected in the IgA^{+} participants (Fig. 2E, F). Moreover, anti-Spike/RBD IgA and the level of secretory component positively correlated with each other (Supplementary Fig. 4). Combined with the finding that most participants did not produce IgM Ab to anti-Spike/RBD (data not shown), we conclude that secretory component is associating with anti-Spike/RBD IgA (SIgA). Therefore, a local SIgA response to Spike/RBD is produced in response to mRNA vaccination in some participants.

Decay kinetics of anti-Spike/RBD IgG and IgA and neutralizing activity in the saliva of COVID-19 mRNA vaccinated participants

Since the LTCH workers were the earliest recipients of mRNA vaccines during the Canadian roll-out, we had the opportunity to examine the stability of anti-Spike/RBD IgG and IgA in the saliva of a subset of the LTCH cohort who had reached 6 months post-dose 2. We examined the level of salivary Ab only in those participants who had remained anti-Spike IgA positive at 2 weeks post-dose 2 ($n = 32$, ~30% of participants). Using a paired analysis, we observed a significant decline in antigen-specific IgG and IgA levels in the saliva at this time point compared to 2 weeks post-dose 2 (Fig. 3A–D).

Although low, anti-Spike Ab associated with the secretory component remained stable in both BNT162b2 and mRNA-1273 vaccinated subjects, anti-RBD secretory component-associated antibodies were significantly reduced at 6 months post-dose 2 (Fig. 3E, F).

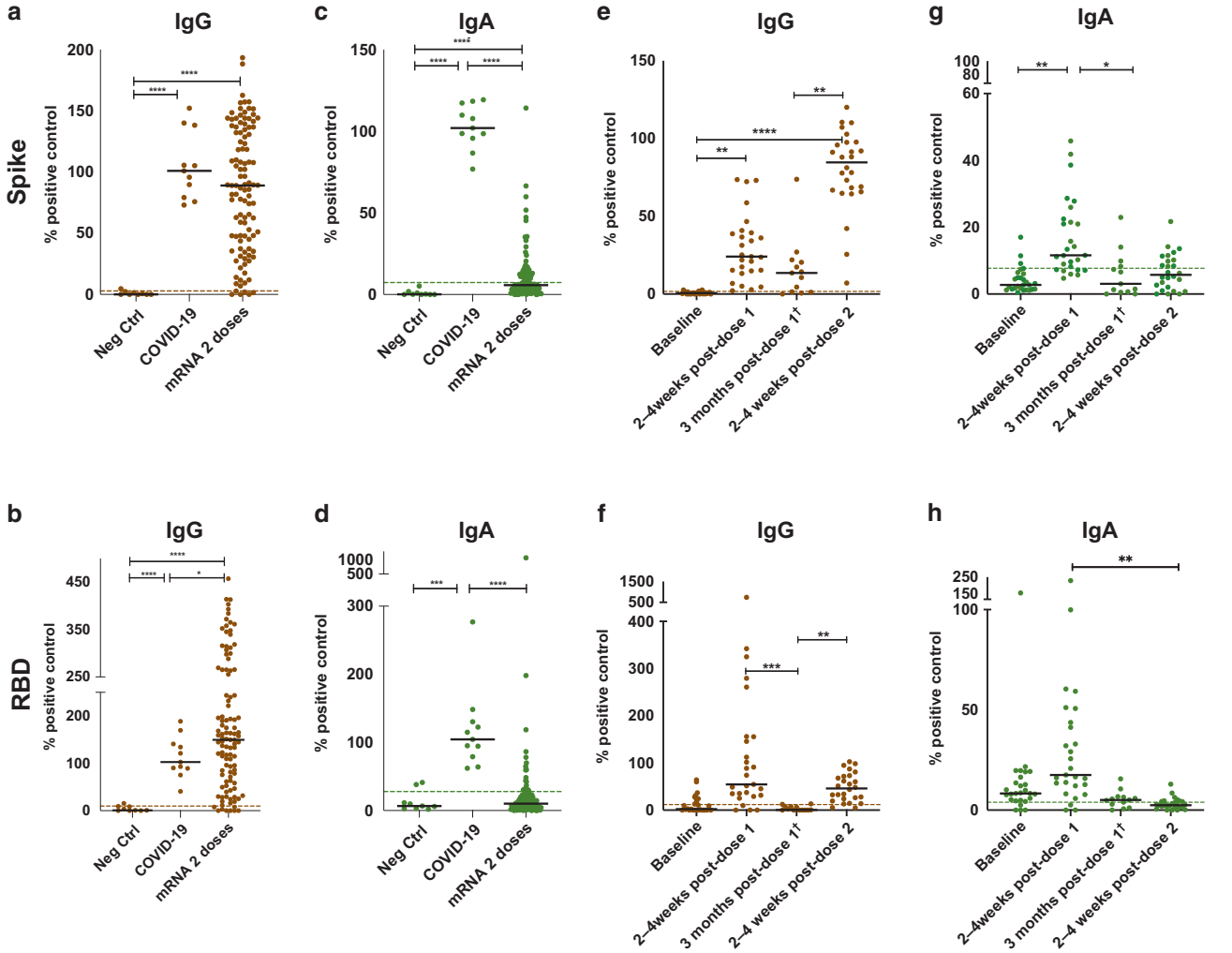

Fig. 1 Analysis of anti-Spike and anti-RBD antibodies in saliva from participants receiving COVID-19 mRNA vaccines. A–D Anti-Spike/RBD IgG and IgA were measured in the saliva of vaccinated LTCH workers after two doses of mRNA vaccine ($n = 107$) and compared to COVID-19 convalescent controls ($n = 11$) and individually run negative controls ($n = 9$). The positive cutoff (dotted line) was calculated as 2 standard deviations above the mean of a pool of negative control samples ($n = 51$) for each individual assay. **E–H** Anti-Spike/RBD IgG and IgA were also analyzed in the saliva of vaccinated participants that were followed at sequential timepoints before and after dose 1 and dose 2 of mRNA vaccination (MSB-1, $n = 27$, with $n = 13$ participants at 3 months post-dose 1, and $n = 26$ at 2 weeks post-dose 2). All data are expressed as a percentage of a pooled positive plate control of $n = 11$ COVID-19 convalescent saliva (see Methods). Solid black bars denote the median for each cohort. Mann–Whitney *U* test was used to calculate significance for (**A–D**). For (**E–H**) a Friedman pair-wise multiple comparison test was used for pair-wise comparisons for multiple timepoints, considering only data points from participants who had completed all timepoints ($n = 11$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. † = we were only able to recall $n = 13$ of the participants at 3 months post-dose 1.

In addition, we assessed if COVID-19 mRNA vaccination provokes SARS-CoV-2 neutralizing activity in the oral cavity, and if this would also be subject to decay. Specifically, saliva at two-fold dilutions was added to hACE2-mCherry expressing HEK293 cells that were co-incubated with recombinant Vesicular Stomatitis Virus (rVSV)-eGFP-SARS-CoV-2-Spike. Infection of HEK293 cells was measured by fluorescence over the course of 72 h, with prevention of infection by added saliva read out as a green fluorescence reduction in neutralization titer (FRNT). We observed that although saliva from LTCH participants taken at 2–4 weeks post-dose 2 had variable capacity to prevent viral entry into $hACE2^+$ HEK293 cells, this was significantly reduced at 6 months post-dose 2 (Supplementary Fig. 5).

Taken together, with the exception of Spike-specfic SIgA, most Ab against SARS-CoV-2 in the saliva, as well as neutralizing capacity, significantly decline over a 6-month period.

Participants who experience a breakthrough infection have lower levels of vaccination-induced anti-Spike IgA

Correlates of protection against SARS-CoV-2 breakthrough infection are ill-described and have only been examined for IgG[10]. Given that we have detected anti-Spike/RBD IgA in the serum and saliva of mRNA vaccinated participants, we examined whether IgA levels may be associated with protection against breakthrough infection. On 20th April 2021, an outbreak of P.1 lineage SARS-CoV-2 (Gamma variant) was declared in a Toronto LTCH home[11]. The residents, who were all doubly vaccinated with mRNA-1273, were part of a separate serum antibody study following LTCH residents that included sampling of blood, but not saliva, at 2–4 weeks post-dose 2 (received on February 2, 2021). Since anti-Spike and anti-RBD IgA levels correlate with each other at 2–4 weeks post-dose 2 (Supplementary Fig. 2), we also measured serum anti-Spike IgA at this time point as a proxy of the salivary

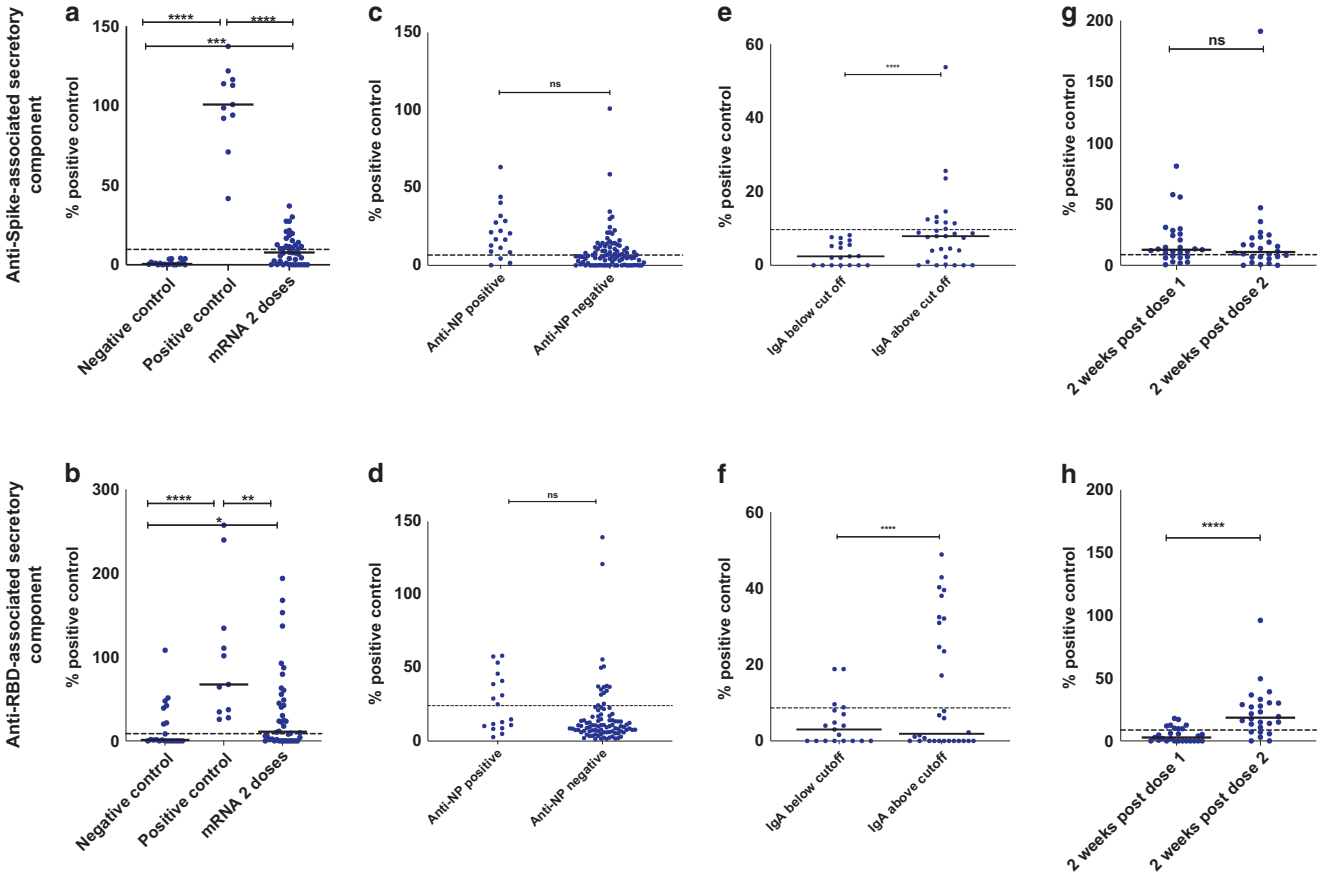

Fig. 2 Detection of secretory component associated with anti-Spike and anti-RBD antibodies in saliva from participants receiving COVID-19 mRNA vaccines. An ELISA-based method was used to detect secretory component associated with anti-Spike (**A**) and anti-RBD (**B**) antibodies in the saliva of 2-dose vaccinated subjects ($n = 42$), as well as saliva taken from COVID-19 negative and positive patients ($n = 21$ and 11, respectively). **C**, **D** Subjects vaccinated with 2 doses of either BNT162b2 or mRNA1273 were grouped based on anti-NP+ ($n = 18$) vs. anti-NP− ($n = 93$) antibody status, which is indicative of previous infection. **E**, **F** 2-dose vaccinated subjects were also subset into those that were considered above ($n = 30$) or below ($n = 19$) the positive cutoff for salivary IgA and analyzed for secretory component. Secretory component associated with anti-Spike (**G**) and anti-RBD (**H**) antibodies was also analyzed in samples collected post-dose 1 ($n = 27$) and post-dose 2 ($n = 26$) from MSB-1. Solid black bars denote the median for each cohort, while the dotted black line denotes the positive cutoff, calculated as 2 standard deviations above the mean of a pool of negative control samples Mann–Whitney *U* test was used to calculate significance, with ns not significant, $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

IgA response. In the context of this isolated outbreak where $n = 5$ residents were infected we did not see a significant difference between exposed infected vs. exposed uninfected participants in terms of levels of anti-Spike and anti-RBD IgG at 2–4 weeks post-dose 2 (Fig. 4A). We noted a trend of reduced anti-Spike and anti-RBD IgA levels in exposed infected vs. exposed uninfected participants that reached significance but did not survive a post hoc multiple test correction (Fig. 4B).

Given the number of breakthrough infections in this isolated outbreak was small, we tested a replication cohort. We therefore measured serum antibody levels at 2–4 weeks post-dose 2 in a larger case–control cohort of healthcare workers (HCW) who had received 2 doses of BNT162b2 at the Sheba Medical Center in Ramat Gan, Israel, with the majority of cases infected with the Alpha SARS-CoV-2 variant[10]. We found that anti-Spike/RBD IgG levels were modestly but not significantly lower in the serum of cases versus controls (Fig. 4C). In contrast, anti-Spike and anti-RBD IgA levels were both significantly lower in cases vs. controls, and this held up to a multiple comparison test (Fig. 4D). Using the more well-powered Sheba cohort, if we convert these values to the BAU/ml using the 20/136 WHO serum standard (Supplementary Table 7), the median level of IgA that is associated with breakthrough cases is 152.78 BAU/ml and 162.31 BAU/ml for anti-Spike and anti-RBD respectively compared to uninfected controls whose median IgA levels were 471.36 BAU/ml and 495.68 BAU/ml for anti-Spike and anti-RBD respectively.

Thus, despite different vaccine regimes (mRNA-1273 vs. BNT162b2), geographical locations (Canada vs. Israel) and viral exposures (Gamma vs. Alpha), in both cohorts anti-Spike/RBD serum IgA but not IgG levels are lower at 2–4 weeks post-dose 2 in participants who subsequently are infected with SARS-CoV-2.

DISCUSSION

In this study we observed robust IgG levels to Spike/RBD in the saliva of participants immunized with BNT162b2 or mRNA-1273 that correlate with the systemic IgG response, and these IgG Ab are significantly diminished at 6 months post-dose 2. A different picture emerges however, for salivary anti-Spike/RBD IgA. In nearly all participants, we observed a modest SIgA response to Spike/RBD in the saliva after a single dose of mRNA vaccine which is maintained in ~30% of participants after dose 2. Moreover, in these subjects, although the SIgA response to Spike is lower than what is observed with SARS-CoV-2 infection, it is more resilient to decay. This preserved SIgA response observed in a minority of vaccinated participants may be very important for preventing breakthrough infections. Indeed, vaccinated participants who subsequently experienced a SARS-CoV-2 infection had

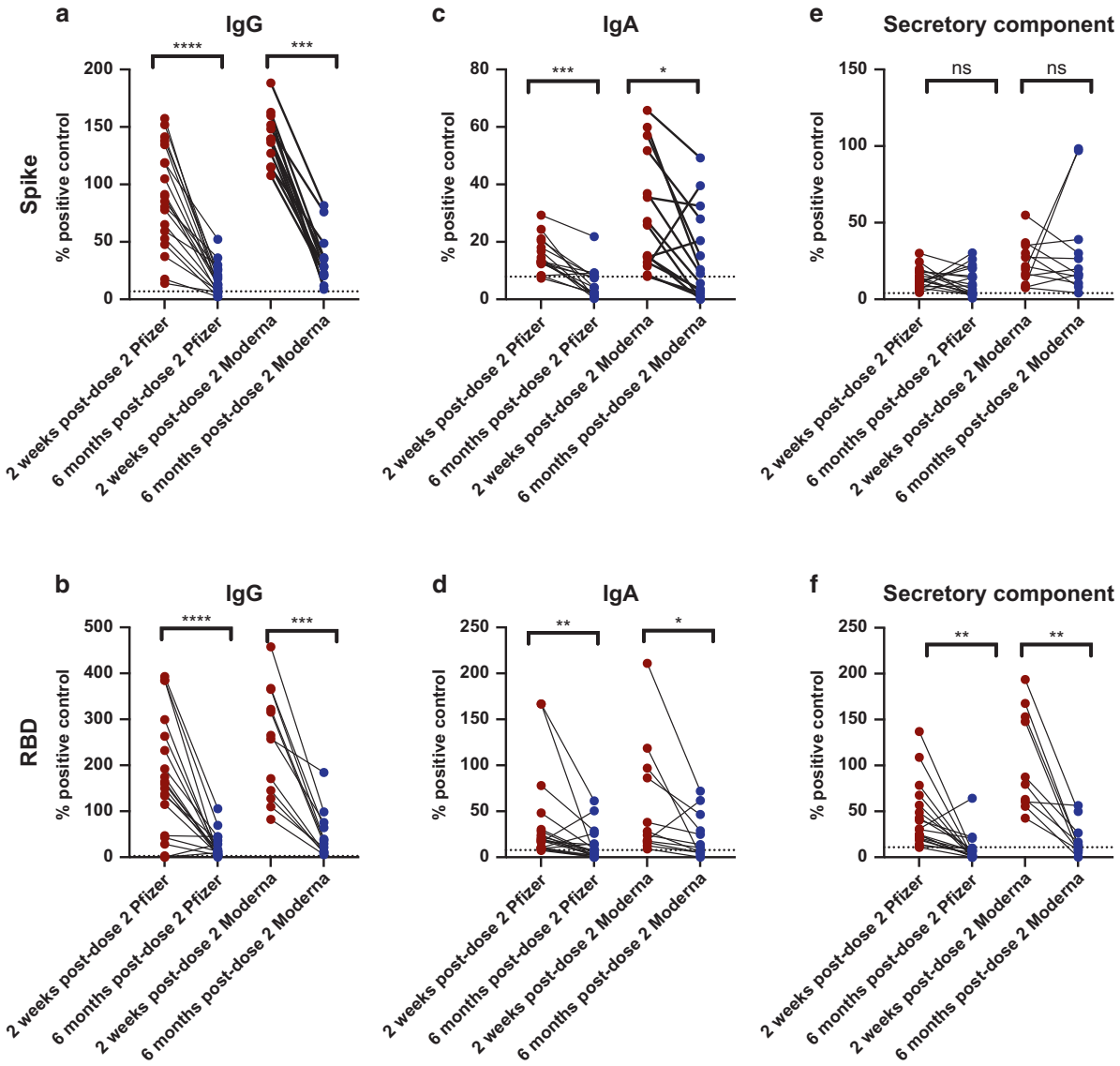

Fig. 3 Different decay kinetics of anti-Spike and anti-RBD IgG versus IgA in saliva from participants receiving COVID-19 mRNA vaccines. Saliva from $n = 32$ LTCH participants was assessed for the presence of IgG (**A**, **B**), IgA (**C**, **D**), and SIgA (**E**, **F**) antibodies against Spike (**A**, **C**, **E**) or RBD (**B**, **D**, **F**). The Wilcoxon signed-rank test was used to calculate significance between groups. ns not significant, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

significantly lower levels of anti-Spike serum IgA at 2–4 weeks post-dose 2 compared to subjects who remain uninfected. Our findings are consistent with recent reports showing that after two doses of BNT162b2 or mRNA-1273, anti-Spike and anti-RBD IgG and IgA could be detected in saliva[12], although infection, or a combination of infection and vaccination, induces more robust[13,14] and durable[15] antigen-specific IgA.

We show evidence of local SIgA production in the saliva by measuring secretory component levels associated with antigen-specific Ab. This is consistent with recent studies measuring anti-Spike IgA in breastmilk of BNT162b2 and mRNA-1273 vaccinated lactating mothers[16]. It is unclear how anti-Spike/RBD SIgA is generated in the saliva following i.m. immunization. Of note, Spike protein can be detected in the plasma, increasing one to 5 days after mRNA-1273 vaccination using an ultra-sensitive detection technique[17]. Thus, one possibility is that plasma-associated Spike antigen may reach the salivary glands (which are surrounded by capillaries[18],) provoking a local SIgA response. Another possibility is that a mucosal IgA response to mRNA vaccination takes place in the gut, and plasma cells generated at this location leave the gut (as we have shown before[19],) disseminating to other mucosal surfaces such as the oral cavity. Indeed, expression of the gut homing integrin (α4β7 on circulating immune cells has been observed following administration of yellow fever[20] and cholera toxin vaccines[21] via the systemic route in humans. Recirculation between mucosal compartments could explain the sustained levels of anti-Spike SIgA in the saliva at 6 months post-boost which is not observed for the systemic IgG response. Animal models are ideal for obtaining further insights into mechanisms that explain how SARS-CoV-2 mRNA vaccines provoke antigen-specific SIgA production at mucosal surfaces.

Dimeric secretory IgA has been shown to have potent neutralization activity against SARS-CoV-2 in vitro[6]. Using a flow cytometry-based pseudovirus entry assay we found that saliva from COVID-19 mRNA vaccinated subjects can variably prevent pseudovirus infection of $ACE2^{+}$ cells. This is consistent with work by Nahass et al. who have examined neutralizing capacity of saliva in response to different vaccine regimes[16]. It is unclear whether

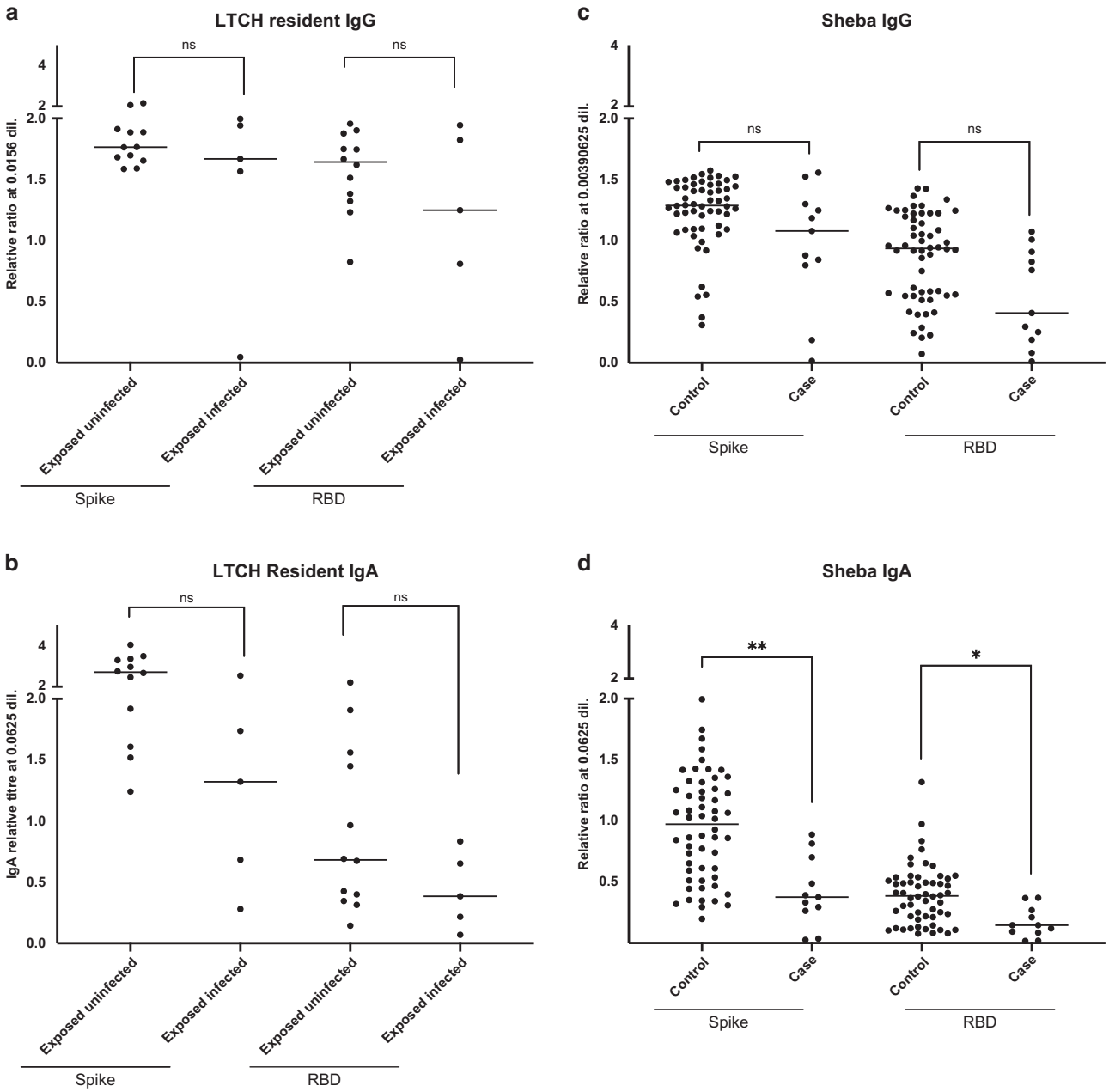

Fig. 4 Participants who experience a breakthrough infection have lower level of anti-Spike/RBD IgA at 2–4 weeks post-vaccination. Serum samples from vaccinated Toronto LTCH residents were taken at 2–4 weeks post-dose 2. Serum anti-Spike/RBD IgG (**A**) and IgA (**B**) levels were compared in participants who were subsequently exposed to P.1 gamma SARS-CoV-2 and either infected (exposed infected, $n = 5$) or not infected (exposed uninfected, $n = 12$). In a separate cohort of double vaccinated healthcare workers from the Sheba Medical Center in Ramat Gan, Israel, serum samples were taken at 2–4 weeks post-dose 2. Serum anti-Spike/RBD IgG (**C**) and IgA (**D**) levels were compared in participants who experienced a breakthrough infection (cases, $n = 11$) vs. controls who did not (control, $n = 56$). Solid black lines denote the median for each cohort. Kruskal–Wallis test with a correction for multiple comparisons was used to conduct statistical analysis between groups. NS not significant, $^{*}p < 0.5$; $^{**}p < 0.01$.

IgG or IgA or both are the main contributors to the neutralizing capacity of saliva from vaccinated subjects. Indeed, although the neutralization activity of saliva at 6 months post-dose 2 was greatly diminished, anti-Spike SIgA levels had not significantly decayed at this time-point. It will be of interest to examine structural interactions between SIgA dimers with SARS-CoV-2 in the context of the mucosa.

There are some limitations to our study: we did not have saliva available from the LTCH residents nor the Sheba HCW for confirmation that the association of high serum anti-Spike IgA levels with protection against breakthrough infection was also observed in a mucosal secretion. We also recognize potential age and health-related differences between these cohorts, as Abe et al. reported LTCH residents had lower nAb and IgG titers in serum compared to LTCH staff[22]. However, our observation that anti-Spike/RBD IgA in the serum and saliva at 2 weeks post-dose 2 correlate (Supplementary Fig. 2), suggests that what we are detecting in the serum at least partially mirrors the oral compartment. Furthermore, we are in the process of collecting saliva samples from the MSB and LTCH cohorts (Fig. 1) that have

experienced Omicron breakthroughs, providing a potential means for assessing correlates of protection against breakthrough infection in the oral compartment.

In summary, we provide evidence that anti-Spike SIgA are induced and maintained in the saliva of approximately 30% of mRNA vaccinated participants and that high anti-Spike/RBD serum IgA levels are associated with protection against subsequent breakthrough infection. Intranasal (i.n.) adenoviral-based vaccines that robustly induce anti-Spike/RBD IgA have been shown to prevent transmission in golden hamsters[23]. Moreover, a SARS-CoV-2 mRNA vaccine i.m. prime followed by an adenoviral i.n. boost provides superior protection against infection compared to i.m. only regimes, even against divergent variants such as B.1.1.351 (beta)[24]. Our data suggest that an i.m. prime followed by i.n. boost strategy is worth considering for preventing person-to-person transmission of SARS-CoV-2 and for broad protection against emerging variants.

MATERIALS AND METHODS

Study approvals

The Mount Sinai Hospital Research Ethics Board (REB) granted approval for recruiting staff in long-term care facilities located in the Greater Toronto Area for blood and saliva collection and for conducting serum ELISAs at the Lunenfeld-Tanenbaum Research Institute (study number: 20-0339-E). The University of Toronto REB granted approval for participant recruitment to collect blood and saliva samples and for conducting saliva ELISAs (study number: 23901). The University of Saskatchewan REB granted approval for saliva sample collection during the pre-COVID era (study number: BIO-USask-1579). The Sheba Medical Center REB granted approval for healthcare worker recruitment (study number: 8008-20-SMC).

Recruitment and participants—LTCH cohort (Supplementary Table 1)

Staff working in Long-Term Care Homes (LTCHs) in Ontario were eligible to participate in the study if: (1) they were over the age of 18, (2) they were comfortable (a) reading and writing in English, (b) providing blood samples, and (c) sharing their COVID-19 diagnostic status with the study team. To invite LTCH staff to participate in the study, study staff approached the administrators and/or directors of LTCHs to assess if they were interested in being a participating site in the study. If they were interested, the administrators and/or directors shared information about the study with their staff and provided a deadline by which staff should opt-out if they did not want to be contacted about the study by a member of the study team. The LTCH administration then shared with the study team the contact information of all LTCH staff who did not opt-out of further communication about the study. The study team then contacted these staff by phone to invite them to participate in the study. This active recruitment strategy was paired with passive recruitment strategies, including having the participating LTCHs and/or relevant staff organizations share recruitment advertisements about the study, as well as having participants approach study staff when they were on-site for sample collection. A total of 12 LTCHs participated as a site in this study. Across these 12 sites, 647 individuals were invited to participate. Of these 647, a subset of individuals was not able to be reached by phone ($n = 242$, 37%), were not eligible to participate ($n = 105$, 16%), or refused to participate ($n = 131$, 20%). Common reasons for participant ineligibility included being beyond 6 weeks after their 2nd dose of their COVID-19 vaccine or not being interested in receiving a COVID-19 vaccine. The most common reason for refusal to participate was lack of interest in being part of research. Finally, a subset of individuals ($n = 13$) withdrew prior to their first sample collection, resulting in a final sample size of 156 participants.

Recruitment and participants—COVID-19 acute and convalescent participants (Supplementary Table 2b)

Acute and convalescent serum and saliva samples were obtained from patients identified by surveillance of COVID-19 (confirmed by PCR; in- and out-patients) by the Toronto Invasive Bacterial Diseases Network in metropolitan Toronto and the regional municipality of Peel in south-central Ontario, Canada (REB studies #20-044 Unity Health Network, #02-0118-U/05-0016-C, Mount Sinai Hospital). Consecutive consenting patients admitted to four Toronto Invasive Bacterial Disease Network (TIBDN) hospitals were enrolled: these patients had serum and saliva collected at hospital admission, and survivors were asked to submit repeat samples at 4–12 weeks post-symptom onset (PSO). Consecutive out-patients diagnosed at the same 4 hospitals prior to March 15th and on a convenience sample of later days were approached for consent to collect serum and saliva at 4–12 weeks PSO. Patients were interviewed and patient charts reviewed to determine age, sex, symptom onset date, and disease severity, as described in[3].

Recruitment and participants—Medical Sciences Building (MSB) (Supplementary Table 4)

Pre-vaccination baseline, 2–4 weeks post-dose 1, 3 months post-dose 1- and 2–4 weeks post-dose 2 serum and saliva samples were obtained from an independently recruited cohort at the University of Toronto in Toronto, Ontario, Canada under REB protocol 23901. Of the $n = 29$ recruited participants, 1 was excluded based on prior COVID-19 exposure, 1 left the study after the first sampling, and 1 left the study after the second sampling (both moved out of the country). For the 3 months post-dose 1 time point, 13 out of the 27 eligible participants provided samples, whereas all other timepoints had either $n = 27$ (baseline + 2 weeks post-dose 1) or $n = 26$ participants (2 weeks post-dose 2). Upon arrival at the sampling site, participants gave informed consent, and serum and saliva samples were collected.

Recruitment and participants—Long-Term Care (LTCH) resident breakthrough cohort (Supplementary Table 5)

Residents in this LTCH were offered mRNA-1273 on 4 January and 2 February 2021. The facility has resident rooms on the second through fifth floors and administrative/service areas in the basement and ground floors. By 28 March, 100 of 124 residents (81%) and 120 of 224 staff (54%) had received 2 vaccine doses, and 7 residents (6%) and 29 staff (13%) had received 1 dose. Ongoing pandemic measures included no visitors except essential caregivers, symptom surveillance twice daily for residents and staff/caregivers, staff/caregiver nasopharyngeal swab SARS-CoV-2 testing 2–3 times weekly by reverse-transcriptase polymerase chain reaction (rt-PCR) or daily rapid antigen testing (Panbio), cohorting of staff by floor to limit any exposure to a single floor, and universal masking and eye protection for staff/caregivers. All positive rapid antigen tests were confirmed by SARS-CoV-2 PCR. All specimens PCR positive for SARS-CoV-2 were tested for N501Y and E484K mutations using a multiplex real-time PCR assay. Public Health Ontario Laboratory and/or Sunnybrook Health Sciences Center performed whole-genome sequencing and assessed predicted Pango lineage, as described elsewhere[25]. On the 2 outbreak units, positive specimens with concentrations too low for mutation detection were assumed to be lineage P.1.

Recruitment and participants—Sheba Health Care Workers breakthrough cohort (Supplementary Table 6)

Beginning January 20, 2021, 11 days after the first staff members had received a second dose of the BNT162b2 vaccine, a study was initiated to identify every breakthrough infection, including asymptomatic infections. Serum analysed herein was collected at ~2–4 weeks post-dose 2. Data were collected until April 28, 2021. Efforts were extended to identify new cases with the use of daily health questionnaires, a telephone hotline, extensive

epidemiologic investigations of exposure events, and contact tracing of infected patients and personnel. Testing for the presence of SARS-CoV-2 by RT-PCR was performed on fully vaccinated staff members who were symptomatic or had been exposed to an infected person, regardless of symptoms. Antigen-detecting rapid diagnostic testing (Ag-RDT) was available as an initial screening tool in the personnel clinic in combination with RT-PCR testing. A breakthrough infection was defined as the detection of SARS-CoV-2 on RT-PCR assay performed 11 or more days after receipt of a second dose of BNT162b2 if no explicit exposure or symptoms had been reported during the first 6 days. A matched case–control analysis was used to identify possible correlates of breakthrough infection. For the case–control analysis, control serum samples were selected from a prospective cohort study to analyze vaccine-induced immune responses and dynamics at the Sheba Medical Center. Each breakthrough case was matched with control samples that had been obtained from uninfected controls according to the following variables: sex, age, immunosuppression status, and the interval between the second dose of BNT162b2 vaccine and serology at the peri-infection timepoint (see ref. [10]) We compared antibody titers of cases versus controls obtained during the initial post-vaccination period. Breakthrough cases for which serologic samples were not available were excluded from this analysis.

Saliva collection

LTCH, MSB-1 and MSB-2 participants were told not to eat, drink or smoke at least 30 min prior to collection. Subsequently, saliva were collected using Salivette® tubes (Sarstedt, Numbrecht, Germany), a collection system which consists of a cotton ball which participants chew for exactly 3 min and place into a tube, which is then placed into a larger outer tube. The entire system is spun in a centrifuge at $1000 \times g$ for 5 min at room temperature. The inner tube contains a hole at the bottom, which allows all the saliva absorbed by the cotton ball to filter into the larger outer tube. The total saliva volume from each participant was then separated into 300–500 µl aliquots and stored at −80 °C until the time of testing. Given that these samples were collected from vaccinated participants who reported no symptoms of COVID-19 infection, we did not conduct any measures for viral inactivation.

Antigen production—Saliva assay

The expression, purification and biotinylation of the SARS-CoV-2 RBD and Spike ectodomain were performed as recently described[3]. The human codon optimized cDNA of the SARS-CoV-2 Spike protein was purchased from Genscript (MC_0101081). The soluble RBD (residues 328–528, RFPN…CGPK) was expressed as a fusion protein containing a C-terminal 6 × His tag followed by an AviTag. The soluble trimeric Spike protein ectodomain (residues 1–1211, MFVF…QYIK) was expressed with a C-terminal phage foldon trimerization motif followed by a 6 × His tag and an AviTag. To help stabilize the Spike trimer in its prefusion conformation, residues 682–685 (RRAR) were mutated to SSAS to remove the furin cleavage site and residues 986 and 987 (KV) were each mutated to a proline residue (***51***). Stably transfected FreeStyle 293-F cells secreting the RBD and soluble Spike trimer were generated using a previously reported piggyBac transposon-based mammalian cell expression system (***52***). Protein production was scaled up in 1 L shake flasks containing 300 mL FreeStyle 293 medium. At a cell density of 10^6 cells/mL, 1 µg/mL doxycycline and 1 µg/mL Aprotinin were added. Every other day 150 mL of medium was removed and replaced by fresh medium. The collected medium was centrifuged at $10000 \times g$ to remove the cells and debris and the His-tagged proteins were purified by Ni-NTA chromatography. The eluted protein was stored in PBS containing 300 mM imidazole, 0.1% (v/v) protease inhibitor cocktail (Sigma, P-8849) and 40% glycerol at −12 °C. Shortly before use, the RBD and Spike proteins were further purified by size-exclusion chromatography on a Superdex 200 Increase (GE healthcare) or Superose 6 Increase (GE healthcare) column, respectively. Purity was confirmed by SDS-PAGE. For the Spike protein, negative stain electron microscopy was used show evidence of high-quality trimers. The Avi-tagged proteins, at a concentration of 100 µM or less, were biotinylated in reaction mixtures containing 200 µM biotin, 500 µM ATP, 500 µM MgCl2, 30 µg/mL BirA, 0.1% (v/v) protease inhibitor cocktail. The mixture was incubated at 30 °C for 2 h followed by size-exclusion chromatography to remove unreacted biotin.

Enzyme-linked immunosorbent assays for detecting anti-Spike and anti-RBD IgA and IgG in saliva

96-well plates pre-coated with streptavidin were used for all saliva assays. We have previously determined that coating plates with 50 µl per well of 2 µg/ml of biotinylated RBD or 20 µg/ml of biotinylated Spike diluted in sterile phosphate-buffered saline (PBS) was the ideal coating solution. Control wells were coated with 50ul per well of sterile PBS. After coating with the antigen of interest and incubating overnight at 4 °C, the coating solution was discarded and plates were blocked with 5% BLOTTO solution (5% w/vol skim milk powder (BioShop, CAT# SKI400.500)). Plates were incubated with the blocking solution at 37 °C for 2 h, and the solution was discarded immediately prior to adding samples to each well. During the blocking incubation, frozen saliva samples were removed from −80 °C storage, thawed and diluted using 2.5% BLOTTO at a range of 1:5–1:20. Sample dilutions were pre-incubated in a separate streptavidin-coated plate with no antigen to reduce anti-streptavidin activity in the saliva.

Samples were incubated in the pre-adsorption plate for 30 min at 37 °C, after which 50 µl of each sample from the pre-adsorption plate was transferred to the corresponding wells of the antigen-coated plates and incubated for 2 h at 37 °C. Next, samples were discarded from the antigen-coated plates, and the plates were washed 3× with PBS + 0.05% Tween 20 (PBS-T (BioShop, CAT# TWN510)). 50 µl of Horse radish peroxidase (HRP)-conjugated goat anti-human-IgG and IgA secondary antibodies (Southern Biotech, IgG: 2044-05, IgA: 2053-05) were added to the appropriate wells at dilutions of 1:1000 and 1:2000 in 2.5% BLOTTO, respectively, and incubated for 1 h at 37 °C. Plate development was done by adding 50 µL of 3,3′,5,5′tetramethylbenzidine (TMB) Substrate Solution (ThermoFisher, 00-4021-56) to each well. The reaction was then stopped by adding 50 µl/well of 1 N H2SO4, and optical density (OD) was read at a wavelength of 450 nm (OD450) on a spectrophotometer (Thermo Multiskan FC). For each sample, the raw OD450 for the PBS control well was subtracted from the raw antigen-specific OD450 value for each sample, at each sample dilution (1:5, 1:10, 1:20). The adjusted OD450 value for each sample dilution (1:5, 1:10, 1:20) was used to calculate the area under the curve (AUC) for each individual sample. The sample AUC was then normalized to the AUC of the positive control, which consisted of saliva collected from COVID-19 acute and convalescent participants. The normalized AUC was multiplied by 100 to give a final percentage, which we deemed the "% of positive control". Each plate also included 1–3 negative controls (with a total of $n = 78$ negative controls used between all experiments presented), which consisted of pre-COVID era saliva incubated in antigen-coated wells. Integrated scores were calculated for all negative control samples, using the same calculation method used for cohort 1 and 2 samples. "Positive" cutoff values for each antigen-specific isotype were calculated using the following formula: average integrated scores of negative samples + 2 (standard deviation of negative control integrated scores). Samples whose score was above the resulting cutoff for each antigen-specific isotype was used to determine which samples from cohorts 1 and 2 had detectable antibody levels in their saliva.

ELISA for detection of secretory component-associated anti-Spike/RBD antibodies

Secretory component-associated antibodies were detected by modifying our saliva Spike/RBD ELISA by using an HRP-conjugated Goat anti-human secretory component detection reagent at a dilution of 1:750 from Nordic MUBio (Cat# GAHu/SC/PO).

Enzyme-linked immunosorbent assays for detecting anti-Spike and anti-RBD IgA and IgG in serum

An automated chemiluminescent ELISA assay was used to analyze the levels of IgG and IgA antibodies to the Spike trimer, and its RBD with the following modifications: RBD (PRO1151, 20 ng/well) antigens were produced in CHO (Chinese Hamster Ovary) cells, and were a kind gift from Dr. Yves Durocher, National Research Council of Canada (NRC). The secondary antibody for IgG was an IgG-HRP fusion (PRO1146, 1:6700 or 0.9 ng/well), donated by the NRC. A standard curve of the VHH72 monoclonal antibody[26] fused to a human IgG1 Fc domain (PRO23, also from the NRC) was generated for calibrating the anti-Spike and anti-RBD IgG response. All other antigens, detection reagents and calibration reagents were as previously described[27]. The data analysis also proceeded as in[27], with the following exceptions: Blanks were not subtracted from the chemiluminescence raw values of the samples, and the raw values were normalized to a blank-subtracted point in the linear range of the calibration standard curve (for Spike and RBD, the reference point was 0.0156 µg/ml and for N either 0.0625 µg/ml or a combination of 0.0156 and 0.0312 µg/ml was used to maintain consistent values across tests that used different anti-N antibody batches). The results are represented as a "relative ratio" to this reference point. To define the cutoff for positive antibody calls for each antigen for IgG when 0.0625 µl/well of sample was added, 3 standard deviations from the mean of the log negative control distribution from >20 different runs collected over 4 months was used. For IgA, negatives from 2 different runs over one month were used. In all cases, the selected cut offs correspond to <2% False Positive Rate (FPR) assessment, based on Receiver Operating Characteristic Curves.

Calibration to the WHO standard for serum IgA detection

For anti-Spike and anti-RBD IgA, the relative ratio from the WHO International Standard (IS, National Institute for Biological Standards and Control (NIBSC, South Mimms, United Kingdom), Code 20/136, pooled convalescent plasma) at different sample dilutions (in binding antibody units (BAU)/ml) was represented in log-log scale. The response curve was modeled by an S-shaped sigmoid curve ($y = a*x/(1 + b*x) + c$, expressed in log(y), log(x) coordinates). The nls function (nonlinear least squares) from the R stats package was used to best match the response to the measured data. The interval where the log response was considered linear to the log of the BAU/ml was selected visually. The lm (linear model) function from the R stats package was used to obtain the parameters of the linear approximation of the curve. Estimated BAU/ml values were obtained from the above linear approximation using only sample dilutions that were within this linear range, and further adjusted by accounting for the dilution factor. To convert from relative ratios (RRs) to BAU/m1 for plasma or serum samples where only the reference curve was included in the same test, a conversion formula can be applied: log2 (sample BAU/ml at dilution fold d) = (log2 (sample RR) − a)/b + log2 (d) where a and b represent the y-intercept and slope of the linear interval of the IS curve. Please see Supplementary Table 7 for conversion table and ref. [27].

Flow cytometry method for detection of neutralizing activity

Neutralizing activity was measured at two step saliva dilutions (1:12-1:384) following incubation with recombinant Vesicular Stomatitis Virus (rVSV)-eGFP-SARS-CoV-2-Spike in which the VSV-G protein was replaced with SARS-CoV-2-Spike protein was propagated on MA104 cells[28]. MA104 cells were maintained in Medium 199 (Gibco, Cat. No. 11150067) supplemented with 10% FBS and 1% Penicillin/Streptomycin (Fisher Scientific, Cat. No. 15-140-163). After visible cytopathic affect, supernatant was filtered, aliquoted and stored at −80 °C. Supernatant was added to Human Embryonic Kidney (HEK) 293 cells were engineered to encode human angiotensin converting enzyme 2 (hACE2 in the pDEST-mCherry vector) as previously described[29]. HEK293-hACE2-mCherry cells were cultured in Gibco Dulbecco's Modified Eagle Medium (DMEM) formulation containing glucose, L-glutamine and sodium pyruvate (Gibco, Cat. No. 11995065) with 1× Penicillin/Streptomycin. Geneticin Selective Antibiotic (G418) (Gibco, Cat. No. 10131035) was added at a concentration of 500 µg/mL to maintain hACE2-mCherry expression. Cells were grown in 5% carbon dioxide (CO_2) at 37 °C and passaged every 3 days using Versene solution (Gibco, Cat. No. 15040066). HEK293-hACE2-mCherry cells were seeded at a density of 25,000 cells per well in a 96-well, flat-bottom tissue culture coated plate. Outer rows were avoided to reduce assay variations resulting from edge effect in the IncuCyte. In a separate 96-well plate, samples were serially diluted and incubated with 50 µl of rVSV-eGFP-SARS-CoV-2-S for 2 h at 37 °C in 5% CO_2. Each sample plate included a dilution of anti-RBD antibody (Invitrogen, Cat. No. 703958) of 10, 5, 1, 0.5, 0.1, and 0.05 µg/mL as a positive control. After incubation, the mixture of sample and rVSV-eGFP-SARS-CoV-2-S was transferred to the plated HEK293-hACE2-mCherry cells at a 1:1 ratio of culture media to virus/sample suspension. Plates loaded in the IncuCyte were imaged every 1–4 h for a total of 72 h with 4 scans per sample well to visualize neutralization. The total integrated intensity of the fluorescent value of the lowest anti-RBD condition (0.05 µg/mL) with the rVSV-eGFP-SARS-CoV-2-S controls at 12 h intervals was used to normalize separate experiment runs. Each plate was normalized either to the mean of the rVSV-eGFP-SARS-CoV-2-S supernatant controls or to the 0.05 µg/mL anti-RBD antibody. Normalization to 0.05 µg/mL anti-RBD was performed only if division by the triplicate rVSV-eGFP-SARS-CoV-2 control conditions resulted in loss of a sigmoidal shape of the anti-RBD curve. To quantitatively determine assay sensitivity, fluorescent reduction of neutralization titers (FRNT) 50 and 70 were calculated to determine the amount of monoclonal anti-RBD needed to prevent 50% and 30%, respectively, of the maximum infection (Supplementary Fig. 10B, C) and plotted in log10 scale for plasma and log2 scale for saliva due to variations in antibody titers in different tissue types. This assay is sufficiently sensitive down to 5 µg/mL of neutralizing antibodies.

Statistics

Antibody levels from the cross-sectional LTCH cohort were compared between groups using Mann–Whitney *U* tests. For correlation analyses, a nonparametric Spearman correlation test was applied. A Friedman pair-wise multiple comparison test was used to compare participants who were assessed at multiple timepoints (Fig. 1), whereas the Wilcoxon pair-wise signed-rank test was used when examining 2 different timepoints (Fig. 3, Supplementary Fig. 5). Kruskal–Wallis with Dunn's multiple comparison test was conducted to examine the differences in antibody levels to RBD and Spike between infected vs. non-infected participants in the LTCH and Sheba cohorts. Adjusted *P* value based on 2 comparisons per family (RBD case vs. control and Spike case vs. control) were used to determine statistical significance. Analyses were performed in Prism (GraphPad) Version 9.2.0.

Multivariable analysis (Supplementary Table 3a–c)

The relationships between clinical predictors (age, sex, SARS-CoV-2 infection prior to vaccination, and time from vaccination to sample collection) and antibody levels were examined in bivariate and a priori multivariable linear regression models. For saliva, four multivariable linear regression models were constructed to examine

potential independent associations between the four clinical predictors and anti-RBD IgA/IgG and anti-Spike IgA/IgG. Kaplan–Meier survival curve were plotted by dividing each group based on serum titer at above or below median. Log-rank (Mantel–Cox) test was done to compare survival curve of each isotype (IgG and IgA anti-Spike) above and below median cohort values.

REFERENCES

1. Walls, A. C. et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* **181**, 281–292.e286 (2020).
2. Zou, L. et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med.* **382**, 1177–1179 (2020).
3. Isho B. et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci. Immunol.* **5**, eabe5511 (2020).
4. Huang, N. et al. SARS-CoV-2 infection of the oral cavity and saliva. *Nat. Med.* **27**, 892–903 (2021).
5. Stadtmueller B. M. et al. The structure and dynamics of secretory component and its interactions with polymeric immunoglobulins. *Elife* **5**, e10640 (2016).
6. Wang Z. et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Sci. Transl. Med.* **13**, eabf1555 (2021).
7. Sterlin D. et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *MedRxiv* https://www.medrxiv.org/content/10.1101/2020.06.10.20126532v1 (2020).
8. Fischinger, S., Boudreau, C. M., Butler, A. L., Streeck, H. & Alter, G. Sex differences in vaccine-induced humoral immunity. *Semin. Immunopathol.* **41**, 239–249 (2019).
9. Demonbreun, A. R. et al. COVID-19 mRNA vaccination generates greater IgG levels in women compared to men. *J. Infect. Dis.* **224**, 793–797 (2021).
10. Bergwerk, M. et al. Covid-19 breakthrough infections in vaccinated health care workers. *N. Engl. J. Med.* **385**, 1474–1484 (2021).
11. Williams, C. et al. COVID-19 outbreak associated with a SARS-CoV-2 P.1 Lineage in a long-term care home after implementation of a vaccination program—Ontario, April-May 2021. *Clin. Infect. Dis.* **74**, 1085–1088 (2021).
12. Ketas, T. J. et al. Antibody responses to SARS-CoV-2 mRNA vaccines are detectable in saliva. *Pathog. Immun.* **6**, 116–134 (2021).
13. Sano, K. et al. Efficient mucosal antibody response to SARS-CoV-2 vaccination is induced in previously infected individuals. *medRxiv* https://doi.org/10.1101/2021.12.06.21267352 (2021).
14. Azzi, L. et al. Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. *EBioMedicine* **75**, 103788 (2022).
15. Meyer-Arndt, L. et al. Cutting edge: serum but not mucosal antibody responses are associated with pre-existing SARS-CoV-2 spike cross-reactive CD4(+) T cells following BNT162b2 vaccination in the elderly. *J. Immunol.* **208**, 1001–1005 (2022).
16. Nahass, G. R. et al. Intramuscular SARS-CoV-2 vaccines elicit varying degrees of plasma and salivary antibody responses as compared to natural infection *Medrxiv* https://doi.org/10.1101/2021.08.22.21262168 (2021).
17. Ogata, A. F. et al. Circulating SARS-CoV-2 vaccine antigen detected in the plasma of mRNA-1273 vaccine recipients. *Clin. Infect. Dis.* **74**, 715–718 (2022).
18. Zhang, C. Z. et al. Saliva in the diagnosis of diseases. *Int J. Oral. Sci.* **8**, 133–137 (2016).
19. Rojas, O. L. et al. Recirculating Intestinal IgA-producing cells regulate neuroinflammation via IL-10. *Cell* **176**, 610–624.e618 (2019).
20. Masopust, D. et al. Dynamic T cell migration program provides resident memory within intestinal epithelium. *J. Exp. Med.* **207**, 553–564 (2010).
21. Quiding-Jarbrink, M. et al. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. *J. Clin. Investig.* **99**, 1281–1286 (1997).
22. Abe, K. T. et al. Neutralizing antibody responses to SARS-CoV-2 variants in vaccinated Ontario long-term care home residents and workers. *medRxiv* https://doi.org/10.1101/2021.08.06.21261721 (2021).
23. Langel, S. N. et al. Oral and intranasal Ad5 SARS-CoV-2 vaccines decrease disease and viral transmission in a golden hamster model. *bioRxiv* https://doi.org/10.1101/2021.10.03.462919 (2021).
24. Lapuente, D. et al. Protective mucosal immunity against SARS-CoV-2 after heterologous systemic prime-mucosal boost immunization. *Nat. Commun.* **12**, 6871 (2021).
25. Guthrie, J. L. et al. Detection of the novel SARS-CoV-2 European lineage B.1.177 in Ontario, Canada. *J. Clin. Virol.* **1**, 100010 (2021).
26. Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263 (2020).
27. Colwill, K. et al. A scalable serology solution for profiling humoral immune responses to SARS-CoV-2 infection and vaccination. *Clin Transl Immunology* **11**, e1380 (2022).
28. Case, J. B. et al. Neutralizing antibody and soluble ACE2 inhibition of a replication-competent VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. *Cell Host Microbe* **28**, 475–485.e475 (2020).
29. Zang, R. et al. Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. *Proc. Natl Acad. Sci. U.S.A.* **117**, 32105–32113 (2020).

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AUTHOR CONTRIBUTIONS

S.S.M., B.I., and M.Z. performed saliva Ab measurements, prepared figures and were supervised by J.G. G.C. coordinated intake, database entry and maintenance for all samples and also consented and performed phlebotomy on MSB participants and was supervised by J.G. C.C. and Y.L. recruited, consented and collected serum from Sheba healthcare workers and were supervised by G.R. G.R.N., R.E.S.S. and G.B. performed neutralization studies with saliva and were supervised by I.L.W. and M.C.T. M.F.Z., B.R., and K.C. performed serum antibody measurements and were supervised by A.C.G. A.J. performed multivariate analyses and was supervised by A.M. Z.L. purified and prepared biotinylated Spike and RBD proteins and was supervised by J. M.R. K.Q.d.L., A.T., J.G.T., A.P., and C.F. recruited, consented and collected saliva, serum and metadata from LTCH participants at multiple LTCH sites and were supervised by S.S. A.P., A.X.L., N.H., S.B., L.G., K.G., and M.M. recruited, consented and collected saliva from COVID-19 convalescent patients and were supervised by A.M. P.S. and P.B. recruited, consented and collected saliva from COVID-19 convalescent patients and were supervised by M.O. WS recruited, consented and collected saliva from pre-COVID-19 participants. O.L.R. assisted in data analysis. J.G. and S.S.M. wrote the paper.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Sharon Straus, Anne-Claude Gingras or Jennifer L. Gommerman.