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Original article

Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine

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OBJECTIVE: The dynamic adaptive immune responses elicited by the inactivated virus vaccine CoronaVac remain elusive.

METHODS: In a prospective cohort of 100 healthcare professionals naïve for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) who received two doses of CoronaVac, we analysed SARS-CoV-2-specific humoral and cellular responses at four different time points, including before vaccination (T1), 2 weeks after the first dose (T2), 2 weeks after the booster dose (T3), and 8–10 weeks after the booster dose (T4). SARS-CoV-2-specific antibodies, serum neutralizing activities, peripheral B cells, CD4+ and CD8+ T cells and their memory subsets were simultaneously measured in this cohort.

RESULTS: SARS-CoV-2 spike-specific IgG responses reached a peak (geometric mean titre (GMT) 54,827, 30,969–97,065) after two doses and rapidly declined (GMT 502, 212–1190) at T4, whereas suboptimal IgA responses were detected (GMT 5, 2–9). Spike-specific circulating B cells (0.60%, 0.46–0.73% of total B cells) and memory B cells (1.18%, 0.92–1.44% of total memory B cells) were effectively induced at T3 and sustained over time (0.33%, 0.23–0.43%; 0.87%, 0.05–1.67%, respectively). SARS-CoV-2-specific circulating CD4+ T cells (0.57%, 0.47–0.66%) and CD8+ T cells (1.29%, 1.04–1.54%) were detected at T3. At T4, 0.78% (0.43–1.20%) of memory CD4+ T cells and 0.68% (0.29–1.30%) of memory CD8+ T cells were identified as SARS-CoV-2-specific, while 0.62% (0.51–0.75%) of CD4+ T cells and 0.47% (0.38–0.58%) of CD8+ T cells were SARS-CoV-2-specific terminally differentiated effector memory cells. Furthermore, age and interval between doses affected the magnitude of CoronaVac-induced immune responses. SARS-CoV-2 memory CD4+ T cells were strongly associated with both receptor binding domain (RBD)-specific memory B cells (r 0.87, p < 0.0001) and SARS-CoV-2-specific memory CD8+ T cells (r 0.48, p < 0.0001).

CONCLUSIONS: CoronaVac induced robust circulating and memory B cell and T cell responses. Our study offers new insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future.

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coronavirus 2 (SARS-CoV-2), has so far been inoculated into at least 243 million individuals from more than 45 countries. A large, observational study in Chile indicated that two doses of CoronaVac had a vaccine effectiveness of 65.9% against coronavirus disease 2019 (COVID-19), 90.3% against intensive care unit admission and 86.3% against COVID-19-related death [1]. Nevertheless, few studies on CoronaVac recipients focused largely on binding and/or neutralizing antibodies (NAbs) as primary endpoints, while vaccine-induced cellular immune responses remain elusive.

It is well established that three fundamental components of the adaptive immune system (B cells, CD4⁺ and CD8⁺ T cells) are essential to control SARS-CoV-2 infection [2–7]. Despite the immune correlates of protection remaining unknown [8,9], antibodies and T-cell responses are important for the resolution of primary SARS-CoV-2 infection. Additionally, SARS-CoV-2 infection induced various immunological memory components displaying distinct kinetics [10].

Recently, we conducted a prospective, observational cohort study (NCT04729374) with 100 healthcare personnel in a tertiary hospital in Nanjing, China. Most sera elicited by two-dose CoronaVac were capable of effectively neutralize the ancestral strain, Alpha and Epsilon variants, but not Beta and Gamma variants bearing E484K mutation [11]. In this current study, we provided data from this cohort with new insights into the kinetics of vaccine-induced humoral and cellular immune responses, including circulating antibodies, antigen-specific B cells, CD4⁺ and CD8⁺ T cells, as well as their memory subsets at four timepoints extending up to 8–10 weeks post two-dose immunization. The impact of gender, age and interval between doses on the magnitude of vaccine responses were further analysed. The interrelationships between antibody and cellular responses were also evaluated.

Materials and methods

Study cohort and sample collection

A total of 100 healthcare professionals were enrolled in a prospective study (NCT04729374) from January to February 2021 in Nanjing Drum Tower Hospital. All participants tested negative for SARS-CoV-2 infection at screening and provided written informed consent. The clinical trial protocol was approved by the hospital ethics committee (2021-034-01). Two cohorts of COVID-19 convalescent patients were included, and their demographic characteristics are provided in Fig. 1. In the first cohort, serum samples were collected from 26 convalescent patients on a 4-week follow-up visit after hospital discharge, while peripheral blood mononuclear cells (PBMCs) from 12 convalescent patients were collected 16 months after COVID-19 infection in the second cohort.

SARS-CoV-2-specific humoral and cellular responses

The quantification of antigen-specific antibodies against SARS-CoV-2 and serum neutralization activities were performed as previously described [11,12]. Fluorescence-labelled ectodomain of the spike or receptor binding domain (RBD) proteins were used as probes to identify SARS-CoV-2-specific B cells and memory B cells. PBMCs were stimulated with SARS-CoV-2 peptide pools to measure antigen-specific CD4⁺ and CD8⁺ T cells. The details of peptide pools,

![Fig. 1. The study design and the characteristics of participants in our cohort. (A) The study design of our vaccine cohort. (B) The characteristics of three study cohorts used in our study, including the vaccine cohort who received two doses of CoronaVac, the convalescent coronavirus disease 2019 (COVID-19) patient cohort 1 and the convalescent COVID-19 patient cohort 2.](image-url)
conjugation of antibodies, sample staining and statistical analysis were presented in the Supplementary Material.

Results

Study design

One hundred healthcare workers were enrolled in this study; their ages ranged from 23 to 59 years (median 35), and 63 (63%) were female (Fig. 1). All participants finished two doses of CoronaVac; 50 first-dose recipients received the second dose within 14–21 days after the first dose, and 50 received the second dose between 22 and 30 days. To investigate the kinetics of the immune responses following both primary and secondary immunizations, the serum and PBMC samples were collected for immunological analysis at four different timepoints: pre-vaccine baseline (T1), 2 weeks following the first dose (T2), 2 weeks following the second dose (T3), and 8–10 weeks following the second dose (T4).

SARS-CoV-2-specific humoral responses

At baseline, all participants had undetectable levels of IgM, IgG and IgA antibodies specific for the ectodomain of the spike protein (Spiek), nucleocapsid protein (NP) and RBD protein (Fig. 2A–F and Supplementary Material Fig. S1). Two doses of CoronaVac significantly boosted antibody responses achieving the peak level of humoral immunity, and 100% of the participants seroconverted after two doses of immunization. Specifically, 98 vaccinees (98%) were anti-spik IgG-positive (geometric mean titre (GMT) 54827, 30969–97065) and 23 (23%) were IgA-positive (GMT 5.2–9; 85/100) and 29% (29/100) of sera at T3 were able to neutralize the ancestral strain and B.1.617, respectively. The B.1.617 variant was 2.96-fold resistant to neutralization by sera from CoronaVac recipients, compared to the ancestral strain (Fig. 2G). At T4, spike-specific and NP-specific IgG responses declined significantly, and vaccinee sera had a significantly higher anti-spik IgG titre but remarkable lower IgA responses compared to those in convalescent sera (Fig. 2A–F).

SARS-CoV-2-specific B-cell responses

The first dose of CoronaVac induced a significant proportion of spike-specific B cells (0.32%, 0.27–0.38%), which expanded after the second dose (0.60%, 0.46–0.73%) despite no statistical differences, and slightly reduced at T4 (0.33%, 0.23–0.43%) (Fig. 3A). Similarly, the frequency of spike-specific memory B cells at T3 was on average 1.18% (0.92–1.44%) and gradually reduced to 0.87% (0.10–1.63%) at T4. A similar pattern was observed for RBD-specific B cells and memory B cells (Fig. 3B). RBD-specific B cells at T4 correlated with serum titres that achieved 50% pseudovirus neutralization (pNT50) against the D614G variant, B.1.1.7 and B.1.526 (Fig. 3C). Vaccinees displayed comparable magnitudes of spike-specific B cells as well as RBD-specific memory B cells, but lower levels of spike-specific memory B cells and RBD-specific B cells at the T4 timepoint, compared to COVID-19-recovered donors (Fig. 3A,B).

Immunoglobin (Ig) isotypes among the antigen-specific memory B-cell population shifted with time (Fig. 3A,B). After primary immunization, ~23% of RBD-specific memory B cells were IgG+ and ~22% were IgM+. The frequency of IgG+ memory B cells surged to ~45% following the second dose, and slightly increased to ~55% 8–10 weeks after full vaccination. RBD-specific IgA memory B-cell frequency was ~13% at both T2 and T3 and slightly increased to ~22% at T4.

B-cell analyses were extended to in vitro stimulation of memory B cells which differentiate into antibody-secreting cells (ASCs) by ELISPOT assay among a small portion of participants (Fig. 3D). The first dose induced positive spike-specific and RBD-specific B cells in 38.9% (21/54) and 22.2% (12/54) of subjects, respectively. The second dose further boosted spike-specific and RBD-specific antibody-secreting B cells in 57.5% (15/26) and 57.5% (15/26) of subjects, respectively. The frequency of spike-specific and RBD-specific memory B cells was stable at T4, and were detected in 70.2% (33/47) and 87.2% (41/47) of subjects.

The magnitude of SARS-CoV-2-specific CD4+ and CD8+ T-cell responses

SARS-CoV-2-specific T-cell responses were analysed by ex vivo stimulation with SARS-CoV-2 peptide pools covering the most commonly recognized T-cell epitopes [4], including S, M, E, N, ORF3a and ORF7/8 (Supplementary Material Fig. S4). Robust expanded SARS-CoV-2-specific CD4+ T cells were detectable in 61.5% (48/78), 74.2% (69/93) and 75.0% (60/80) of the subjects at T2, T3 and T4, respectively (Fig. 4A). SARS-CoV-2-specific CD4+ T-cell responses were also significantly elevated after the primary immunization (0.57%, 0.47–0.66%) compared to that at T1 (0.08%, 0.02–0.27%). The specific CD4+ T cells (0.83%, 0.67–1.00%) elicited after two doses remained stable at T4 (1.22%, 0.98–1.48%)

Minimal circulating SARS-CoV-2 CD8+ T-cell responses (0.06%, 0.05–0.07%) were detected at T1 baseline (Fig. 4B); 80% (52/65) of participants had detectable SARS-CoV-2 CD8+ T-cell responses (0.69%, 0.54–0.84%) at T2. The boosting immunization induced 83.9% (78/93) of subjects with positive SARS-CoV-2 CD8+ T-cell responses (1.29%, 1.04–1.54%), which steadily increased to 1.61% (1.21–2.02%) at T4. Spike-specific CD4+ or CD8+ T cells displayed a similar kinetic to the SARS-CoV-2-specific CD4+ or CD8+ T cells. Interestingly, CoronaVac also induced CD4+ and CD8+ T-cell responses specific to HCoV-OC43 and HCoV-299E spike glycoprotein (Supplementary Material Fig. S5).

At T4, 0.78% (0.43–1.20%) of memory CD4+ T cells and 0.68% (0.29–1.30%) of memory CD8+ T cells were identified as SARS-CoV-2-specific (Fig. 4C). Vaccinees had similar magnitudes of SARS-CoV-2-specific memory CD4+ T cells, CD8+ T cells and spike-specific memory CD4+ T cells, but a lower level of spike-specific memory CD8+ T cells, compared to convalescent donors. The majority of virus-specific CD8+ T cells were identified as CD45RA+CCR7+ effector memory (TEM) or CD45RA+CCR7+ terminally differentiated effector (TEMRA) [13,14]. Among vaccinees at T4, 0.62% (0.51–0.75%) and 0.43% (0.30–0.57%) of CD4+ T cells were SARS-CoV-2-specific TEMRA and TEM, respectively (Fig. 4D), whereas 0.48% (0.38–0.58%) and 0.79% (0.66–0.92%) of CD8+ T cells were SARS-CoV-2-specific TEMRA and TEM, respectively (Fig. 4E). Convalescent patients displayed a similar proportion of virus-specific TEMRA and TEM as the vaccinees. Our data suggest that CoronaVac effectively induced virus-specific memory CD4+ T cells and CD8+ T cells as well as effector populations.

Factors associated with adaptive responses to SARS-CoV-2 inactivated virus vaccine

There were no relationships identified between gender and the magnitude of SARS-CoV-2-specific adaptive responses (Fig. 5A). Consistent with a previous report [15], the participants between 20 and 40 years old had significantly higher neutralizing titres (GMT 42, 33–52) against the ancestral strain, compared to the participants between 40 and 60 years old (GMT 26, 19–37) (Fig. 5B and Supplementary Material Fig. S6A). Despite the fact that young participants had a higher magnitude of serum neutralizing activities than elder individuals, both groups had a comparable level of anti-spik IgG, suggesting potential qualitative differences in spike-
Fig. 2. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody responses following CoronaVac immunization. (A–F) Dynamic antibody titres for (A) anti-spike IgM, (B) anti-spike IgG, (C) anti-spike IgA, (D) anti-NP IgM, (E) anti-NP IgG, and (F) anti-NP IgA at four time points were analysed, including baseline (T1), 2 weeks after the first dose of CoronaVac (T2), 2 weeks post the booster dose (T3), and 8–10 weeks after the booster dose (T4). In addition, the antigen-specific titres were also compared between sera collected from vaccinees at T4 timepoints and convalescent patient cohort 1 (8–10 weeks post symptom onset). Dotted lines indicate the limit of detection (LOD) for the assay. Statistics were calculated using Wilcoxon matched-pairs signed rank for comparison between timepoints and unpaired Wilcoxon test for comparison between vaccinees at T4 and convalescent patients from cohort 1. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns, no significant difference.
Fig. 3. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating B cell and memory B cell responses following CoronaVac immunization. (A) Frequency of spike$^+$ B cells and spike$^+$ memory B cells over time in vaccinees. Frequency of spike$^+$ B cells or memory spike$^+$ B cells was compared between vaccinees at the T4 timepoint and coronavirus disease 2019 (COVID-19) convalescent patients from cohort 2. Proportion of IgG and IgM isotypes over time was determined in spike-specific circulating B cells or memory B cell compartments. (B) Frequency of RBD$^+$ B cells and RBD$^+$ memory B cells over time in vaccinees. Frequency of RBD$^+$ B cells or memory RBD$^+$ B cells were compared between vaccinees at T4 timepoint and convalescent patients from cohort 2. (C) Association analyses for the frequency of RBD-specific circulating B cells at T4 timepoint and pNT50 against D614G, B.1.1.7, and B.1.526, respectively. $P < 0.05$ was considered to be statistically significant. Statistics were analysed using Wilcoxon matched-pairs signed rank between timepoints. (D) B cell ELISPOT assay for a representative vaccine recipient in our cohort over time (left panel). The frequency of anti-spike and anti-RBD antibody-secreting cells at different time points (right panel). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, no significant difference.
Fig. 4. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating CD4⁺ and CD8⁺ T cell responses following CoronaVac immunization. (A) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) CD4⁺ T cells over time among vaccinees, the magnitude of which at T4 were further compared with that in convalescent patients from cohort 2. (B) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) CD8⁺ T cells over time in vaccinees, the magnitudes of which at T4 were further compared with convalescent patients from cohort 2. (C) Proportion of SARS-CoV-2-specific (left) and spike-specific (right) memory CD4⁺ and memory CD8⁺ T cells among vaccinees at T4 timepoint, convalescent patients in cohort 2 and non-vaccinated healthy subjects. (D-E) Distribution of terminally differentiated effector memory T cells (TEMRA) and effector memory T cells (TEM) in CD4⁺ T cells (D) and CD8⁺ T cells (E) from vaccinees at T4 timepoint and convalescent subjects from cohort 2. Statistics were analysed using Wilcoxon matched-pairs signed rank test between timepoints. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns, no significant difference.
Fig. 5. Association of various factors with vaccine-elicited adaptive responses. (A-C) Serum titres that achieved 50% pseudovirus neutralization (pNT50) against the ancestral strain, the P.1, the B.1.351, the B.1.617.1, anti-spike IgG titre, the frequency of spike-specific memory B cells, the frequency of SARS-CoV-2-specific memory CD4⁺ and CD8⁺ T cells compared with (A) gender, (B) age, and (C) interval between doses. (D) Anti-spike IgG titre, the frequency of spike-specific memory B cells, and the frequency of SARS-CoV-2-specific CD4⁺ and CD8⁺ memory T cells among neutralizing antibody (NAb) responders versus NAb non-responders. (E) Correlation analysis of pNT50 against B.1.1.7 and SARS-CoV-2-specific CD4⁺ T cells at T3 timepoint, correlation analysis of spike-specific memory B cells at T4 and spike-specific CD4⁺ T cells at T2, correlation analysis of RBD⁺ memory B cells at T3 and SARS-CoV-2 memory CD4⁺ T cells at T4, and correlation analysis of SARS-CoV-2-specific memory CD8⁺ T cells at T4 and SARS-CoV-2-specific memory CD4⁺ T cells responses at T4. Statistics were analysed using unpaired Wilcoxon test between groups. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001, ns, no significant difference.
specific humoral immunity. There was no association between age and vaccine-induced cellular responses, including spike-specific memory B cells, virus-specific CD4+ T cells and CD8+ T cells. Our data suggest potentially relevant age-related changes in neutralizing activities but not virus-specific T cell or B cell responses.

Furthermore, the interval between two doses is a critical factor that affects the magnitude of the immune responses. The participants with a dosing interval >21 days had higher neutralizing antibody (NAb) titres against the ancestral strain and B.1.617.1, compared to the group with the interval <21 days (Fig. 5C), which might be associated with the increased anti-spike IgG responses. The interval >21 days also induced a higher percentage of spike-specific B cells, SARS-CoV-2-specific memory CD4+ T cells and CD8+ T cells, compared to the group with an interval <21 days. Consistently, the interval correlated with spike-specific CD4+ T cell responses at T3 (Supplementary Material Fig. S6B).

We also addressed the potential relationship between humoral immunity and cellular immune parameters. NAb responders had a significantly higher level of anti-spike IgG responses compared to NAb non-responders at T3 (Fig. 5D). There is a trend that NAb responders generated higher spike-specific memory B cells among total memory B cells than in NAb non-responders. Of note, NAb non-responders generated comparable levels of SARS-CoV-2-specific memory CD4+ and CD8+ T cells. Additionally, neutralization titres against B.1.17 correlated with SARS-CoV-2-specific CD4+ T cells at T3 (r 0.22, p 0.04), and spike-specific memory B cells at T4 correlated with spike-specific CD4+ T cells at T2 (r 0.29, p 0.03). SARS-CoV-2 memory CD4+ T cells at T4 were strongly associated with both RBD-specific memory B cells at T3 (r 0.87, p <0.0001) as well as SARS-CoV-2-specific memory CD8+ T cells at T4 (r 0.48, p <0.0001) (Fig. 5E).

**Discussion**

Here we provided an extensive characterization of adaptive immune responses specific to SARS-CoV-2 following SARS-CoV-2 inactivated vaccine. Our data are encouraging and fill the gaps in our knowledge of immune responses elicited by CoronaVac. First, we observed robust IgG responses specific to spike, RBD and NP after each dose of CoronaVac. However, these antigen-specific IgG responses decayed rapidly within 6–8 weeks, consistent with observations in COVID-19 patients and vaccinees [12,16]. Such waned antibody responses in COVID-19 patients might be caused by a lack of germinal centre (GC) reaction [17], which is essential to generate long-lived and high-affinity antibody responses. Despite the rapid decline in IgG responses, vaccinees displayed higher spike-specific IgG responses but lower RBD-specific IgG responses 8–10 weeks after full vaccination, compared to convalescent subjects. Additionally, SARS-CoV-2-neutralizing IgA was considered as a critical component of the antiviral immune component [18,19]. Nevertheless, SARS-CoV-2-specific IgA responses are suboptimal among most vaccinee recipients, suggesting that the formulation and delivery approach of next-generation COVID-19 vaccine might be further optimized to induce the mucosal immunity. Besides, the vaccinee sera showed reduced levels of neutralizing ability against B.1.617.1 and other circulating variants, highlighting the urgent need for booster doses beyond the conventional two-dose regimen.

We observed a notable expansion of long-lasting, isotype-switched IgG memory B cells among virus-specific memory B cells following vaccinations, lasting for at least 6–8 weeks. Indeed, SARS-CoV-2 infection-induced memory B cells are durable and long-lived for at least 8 months post disease onset [10,20]. Our data indicate that sustained memory B cells might be important for durability of anti-SARS-CoV-2 immunity and potential recall responses to infection or future boost.

Beyond humoral responses, successful protection against infectious diseases can be accomplished by alternative adaptive immune responses, including CD4+ T cells, CD8+ T cells and their corresponding memory subsets [21,22]. SARS-CoV-2-specific CD4+ T cells and CD8+ T cells were associated with reduced disease severity [4,23]. Potent memory CD4+ and CD8+ T cell responses were also detected from vaccinees, and the magnitudes were comparable to those in convalescent patients. Further, a prominent population of CD4+ and CD8+ memory T cells were biased toward TEMRA and TEM cells. These favourable phenotypes were considered as cytotoxic and long-lived with the potential to respond rapidly to eliminate the infected cells [13,24].

Age and interval might account for the heterogeneity of adaptive immune responses elicited by full vaccination with CoronaVac. As widely observed in COVID-19 patients, age correlated with COVID-19 disease severity, which might be associated with a low percentage of naive CD4+ and CD8+ T cells [23]. Here we also observed a trend that the quality of vaccine-elicited immune response deteriorates with age, especially for neutralizing activities [25]. In addition, the dosing interval >21 days was beneficial for robust SARS-CoV-2-specific adaptive responses. Consistently, extended interval vaccination for BNT162b2 could boost the peak antibody responses in older individuals, which might be critical to further optimize the vaccine regimen for provision of effective and sustained immunity [26].

Very few published datasets compared antigen-specific antibody, B cells, CD8+ T cells and CD4+ T cells following vaccination in the same individuals. For those vaccinees who failed to generate neutralizing antibodies, robust spike-specific memory B cells, SARS-CoV-2 memory CD4+ and CD8+ T cells were detected at a similar magnitude as those in NAb responders. Whether these specific CD4+ and CD8+ T cells could also serve as surrogates for protective immunity remains to be determined. Meanwhile, we also identified SARS-CoV-2 memory CD4+ T cells strongly associated with RBD-specific memory B cells as well as SARS-CoV-2 memory CD8+ T cells, indicating a convergent development of humoral and cellular adaptive immunity.

This study has some limitations. The follow-up observation time in our study was relative short, only extending up to 8–10 weeks post full vaccination. Besides, the alternative function of vaccine-elicited antibody such as antibody-dependent cell-mediated cytotoxicity (ADCC) [27] were not evaluated.

In summary, this study demonstrated multiple compartments of adaptive immunity elicited by an authorized inactivated vaccine in an integrated manner. Our study offers insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future.

**Author contributions**

CW, HS and YC designed the study. YC, YT, YY and YX recruited the patients. JP and JN processed the blood samples. ML, YS and YW performed cellular analysis. TX and MM performed the antibody assay. RH, XY and HS analysed and interpreted the data. YC, SY and JN processed the blood samples. JP and JN performed the antibody assay. RH, XY and HS analysed and interpreted the data. All the authors revised the manuscript. All the authors revised the manuscript.

**Transparency declaration**

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.10.006.

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