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Delayed virus-specific antibody responses associate with COVID-19 mortality

To the Editor,

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread rapidly across the globe and has been declared a pandemic by the World Health Organization.[1] Recent studies observed a robust antibody response against SARS-CoV-2 in patients with COVID-19, even in those discharged patients, supporting the key role of humoral response in limiting SARS-CoV-2 infection.[2-4] However, the association of humoral response, especially antibody production, with clinical outcome of COVID-19 is still unknown.

A total of 149 COVID-19 patients, including 92 survived and 57 deceased patients from Tongji Hospital, were enrolled in this study between February and April 2020. The details of the experimental methods are presented in Appendix S1. No significant difference in age, gender, clinical symptoms, and imaging features was recorded between survived and deceased patients. However, the prevalence of chronic obstructive pulmonary disease and cardiovascular disease was significantly higher in deceased patients than in survived patients (Table S1). In line with previous reports,[5,6] deceased patients demonstrated greater levels of a series of inflammatory markers, including C-reactive protein, procalcitonin, ferritin, interleukin (IL)-1, IL-2 receptor, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α in serum compared to survived patients. In contrast, a dramatically reduced lymphocytes, including $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, and NK cells, were noted in deceased patients in comparison with survived patients (Table S2).[7]

To explore the difference of humoral immune responses between survived and deceased patients, we first detected SARS-CoV-2-specific immunoglobulin (Ig)M and IgG levels in serum. To our surprise, we failed to find any difference in overall levels of SARS-CoV-2-specific IgM and IgG between survived (IgM, median with interquartile range (IQR), 36.91 [12.95-69.46]; IgG, 115.5 [77.12-201.2]) and deceased (IgM, 30.39 [7.348-127.3]; IgG, 106 [51.64-238.3]) patients (Figure 1A). Since a dynamic change of virus-specific IgM and IgG antibodies has been noted in patients with COVID-19,[3] we further stratified the patients into early (≤10 days), middle (11-20 days), late (21-30 days), and end (>30 days) stages of disease according to the time from symptom onset to admission. Cases in the early, middle, late, and end stages were 6, 20, 32, and 34 in survived patients, and were 6, 10, 29, and 12 in deceased patients, respectively. During the first 30 days after symptom onset, there were progressive increases in SARS-CoV-2-specific IgM and IgG antibody levels in both survived and deceased patients (Figure 1B, C). However, IgM showed a slight decrease in the end stage compared to late stage in survived patients (Figure 1B, C). Notably, in the early stage, we observed a significantly higher SARS-CoV-2-specific IgM and IgG levels in survived patients (IgM, 29.47 [17.63-179.3]; IgG, 78.42 [47.42-116.8]) compared to deceased patients (IgM, 3.315 [1.803-7.492]; IgG, 33.60 [4.668-43.07]) (Figure 1B, C). Although the median value of IgM in the end stage (89.53 [29.54-127.3]) in deceased patients was higher than that in survived patients (31.98 [5.813-68.44]), this difference failed to achieve statistical significance (Figure 1B, C). These data suggest that delayed protective SARS-CoV-2-specific IgM and IgG production may be associated with COVID-19 mortality.

Following infection with virus, including SARS-CoV-2, naive B cells develop into memory B cells and antibody-secreting cells (ASCs), which are keys for the rapid production of antibodies.[8] Consistent with the progressive increase of SARS-CoV-2-specific IgG, deceased patients in the end stage group demonstrated a slightly higher frequency of $CD19^+CD27^+CD38^+$ ASCs in $CD19^+$ B cells than those in the early stage group, but failed to achieve statistical significance ($P = .065$) (Figure 1D, E). In contrast, the highest frequency of ASCs was noted in the early stage in survived patients, with a gradually decreased trend (Figure 1D, E). Interestingly, deceased patients demonstrated a significantly lower ASC frequency compared to survived patients in the early stage, whereas ASC frequency in the end stage in deceased patients was significantly higher than that in survived patients (Figure 1D, E). Moreover, we also found that the frequency of ASCs was significantly positively correlated with the levels of both IgM and IgG in deceased patients (Figure 1F). The frequency of ASCs displayed no correlation with the levels of antibodies in survived patients but also showed positive correlation with IgG in total patients (Figure S1).

Follicular helper T (T_{FH}) cells are specialized help providers to B cells, especially supporting the generation of long-lived ASCs and memory B cells for humoral memory.[9] It is now clear that activated circulating T_{FH} (cT_{FH}) cells in blood correlate with T_{FH} differentiation in lymphoid tissues and the increased cT_{FH} cells have been reported in patients with COVID-19.[2,3] We thus assessed the inducible T-cell costimulator $(ICOS)^+$programmed cell death protein 1 $(PD\text{-}1)^+$ cT_{FH} cells in COVID-19 patients in the early stage of disease. The gating strategy for T_{FH} is shown in Figure 2A, as previously described.[3,9] In consistent with ASC response, SARS-CoV-2 infection-induced generation of cT_{FH} cells in the early stage in deceased patients was significantly lower than that in survived patients (Figure 2B). Based on C-X-C chemokine receptor 3 (CXCR3) and C-C motif chemokine receptor 6 (CCR6) expression, circulating T_{FH} and non-T_{FH} can be

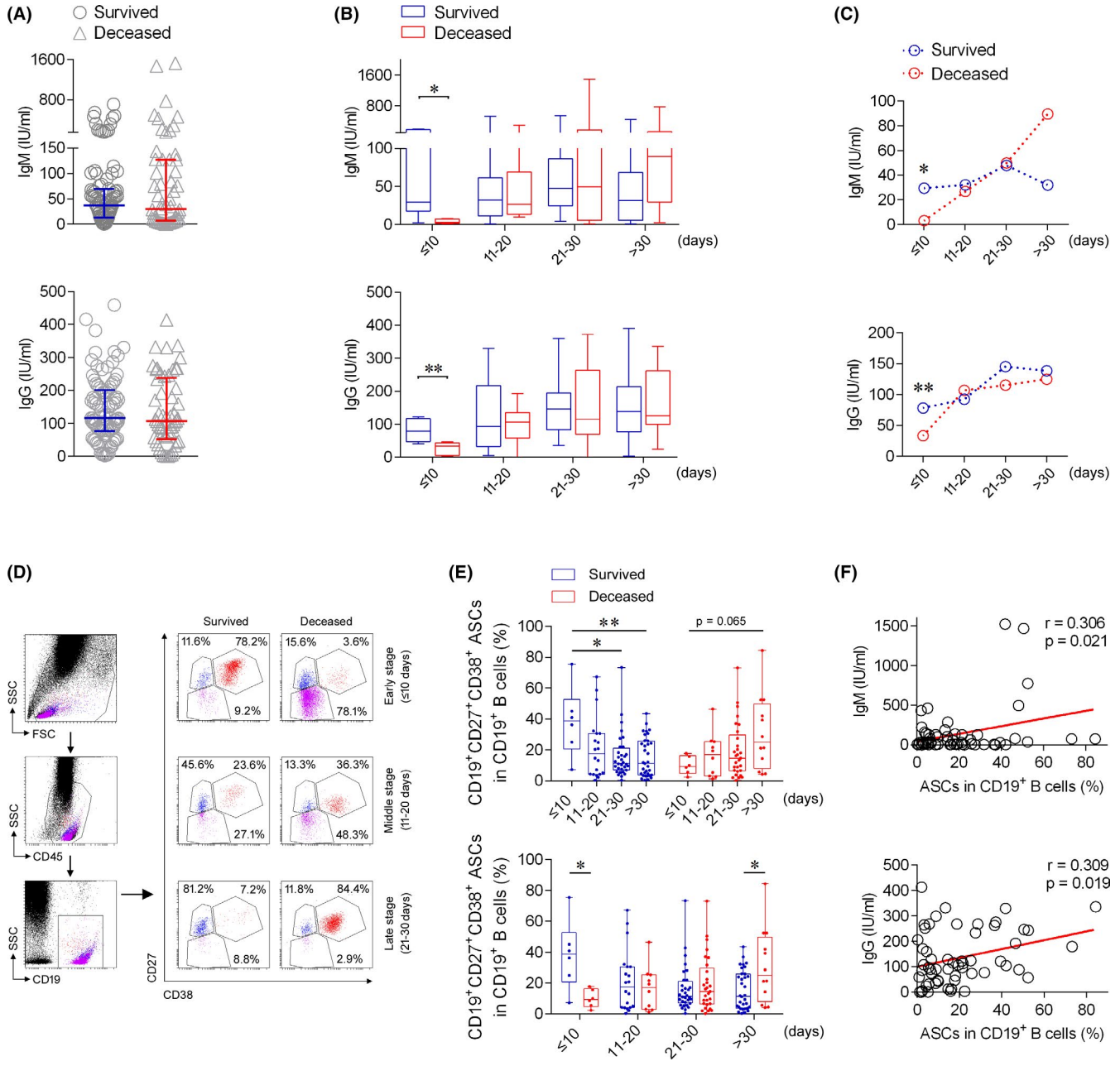

FIGURE 1 SARS-CoV-2-specific antibodies and antibody-secreting cells. A, The levels of SARS-CoV-2-specific IgM and IgG were detected in 92 survived and 57 deceased COVID-19 patients. Data are shown in dot plots and expressed as median with IQR. B, The levels of SARS-CoV-2-specific IgM and IgG in patients with different onset time are shown in box plots. Data are expressed as median with IQR. C, Line graphs showing the median values of IgM and IgG in survived and deceased patients with different onset time. D, Representative FACS plots showing the frequency of $CD19^+CD27^+CD38^+$ ASCs within $CD19^+$ B cells in survived and deceased patients with different onset time. E, The frequencies of ASCs within $CD19^+$ B cells in patients with different onset time are shown in box plots. F, Correlation between SARS-CoV-2-specific antibodies and the percentages of ASCs in 57 deceased patients (Spearman's rank correlation test). $^*P < .05$, $^{**}P < .01$ (Mann-Whitney *U* test)

defined three major subsets: $CXCR3^+CCR6^-$ type 1 ($T_{FH}1$ or T_H1), $CXCR3^-CCR6^-$ type 2 ($T_{FH}2$ or T_H2), and $CXCR3^-CCR6^+$ type 17 ($T_{FH}17$ or T_H17) cells, and $T_{FH}1$ cells have been reported to be associated with neutralizing antibody responses in influenza immunized subjects and hepatitis C virus–infected patients.[10,11] We found that the percentage of $T_{FH}1$ cells, but not $T_{FH}2$ and $T_{FH}17$ cells, was significantly decreased in deceased patients compared with survived patients (Figure 2B). Similarly, the percentage of T_H1 cells, but not T_H2 and T_H17 cells, was also significantly decreased in the early stage of disease in deceased patients compared with survived patients, suggesting a common downregulation of CXCR3 expression on $CD4^+$ T cells in deceased patients (Figure 2B).

Collectively, for the first time, our study provides evidence that delayed antibody responses correlate with poor clinical outcome of

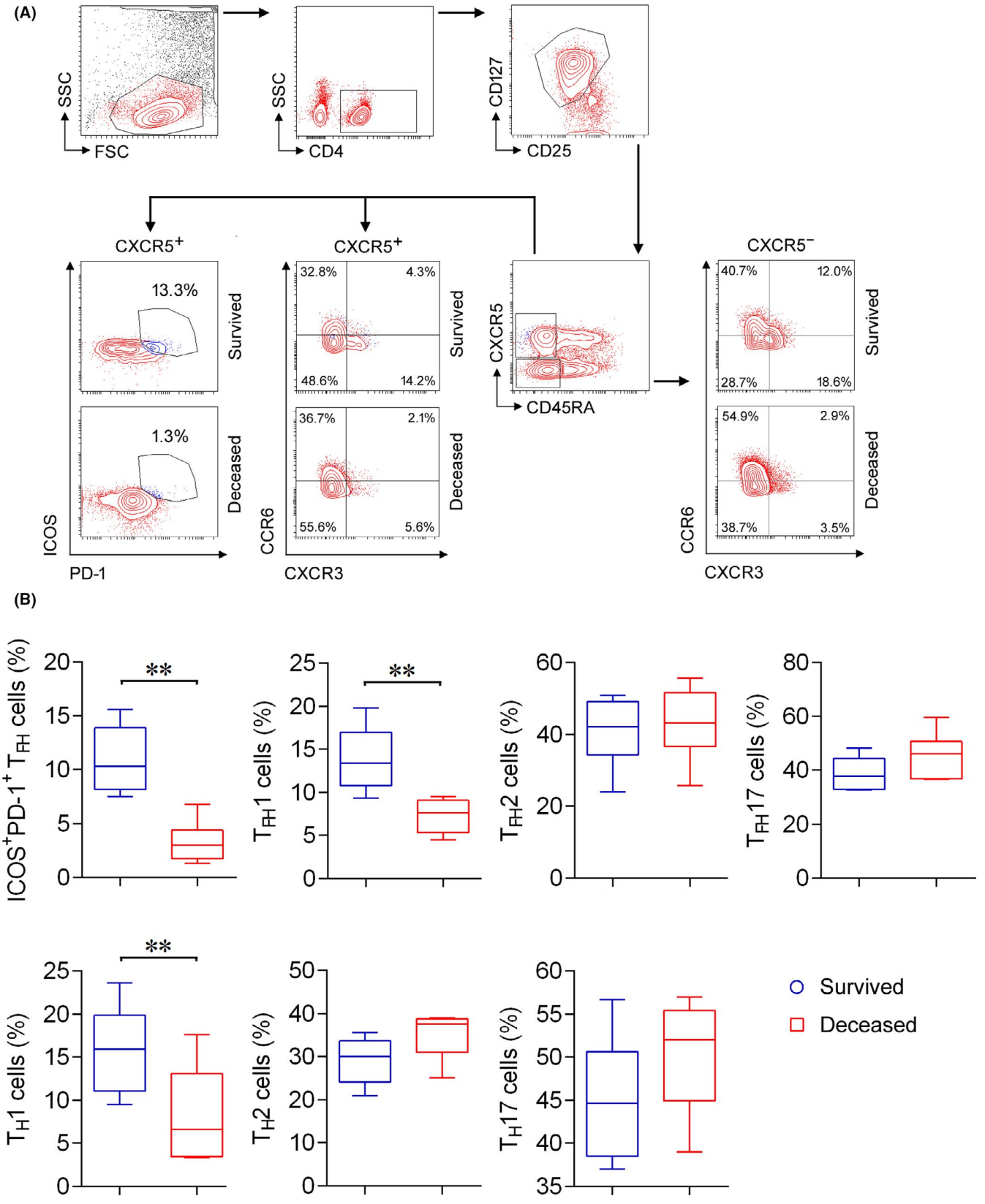

FIGURE 2 T_{FH} and T_H cell subsets in COVID-19 patients. The frequencies of activated T_{FH}, $T_{FH}1$, $T_{FH}2$, $T_{FH}17$, T_H1, T_H2, and T_H17 cells in the early stage in survived and deceased patients were determined. A, Representative FACS plots showing the gating strategies for T_{FH} ($CD4^+CD127^{high}CD25^{low}CD45RA^-CXCR5^+$), activated T_{FH} ($ICOS^+PD\text{-}1^+$ T_{FH}), $T_{FH}1$ ($CXCR3^+CCR6^-$ T_{FH}), $T_{FH}2$ ($CXCR3^-CCR6^-$ T_{FH}), and $T_{FH}17$ ($CXCR3^-CCR6^+$ T_{FH}) cells, and for T_H ($CD4^+CD127^{high}CD25^{low}CD45RA^-CXCR5^-$), T_H1 ($CXCR3^+CCR6^-$ T_H), T_H2 ($CXCR3^-CCR6^-$ T_H), and T_H17 ($CXCR3^-CCR6^+$ T_H) cells. B, The percentages of activated T_{FH}, $T_{FH}1$, $T_{FH}2$, $T_{FH}17$, T_H1, T_H2, and T_H17 cells in survived and deceased COVID-19 patients are shown in box plots. Data are expressed as median with IQR. $^{**}P < .01$ (Mann-Whitney *U* test)

COVID-19 patients. This notion is strongly supported by the reduction of SARS-CoV-2-specific IgM and IgG levels and frequencies of ASCs and T_{FH} cells in the early stage of disease in deceased patients compared with survived patients, which highlights the importance of early adaptive immune responses in patients with COVID-19.

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CONFLICTS OF INTEREST

The authors have declared that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Clinical characteristics of allergic rhinitis patients in 13 metropolitan cities of China

To the Editor,

As an international health problem, allergic rhinitis (AR) currently affects 10%-40% of the global population.[1] Multicenter studies conducted in Europe and America have revealed the clinical features of pediatric, adolescent, and adult patients with AR, including the subtype percentages, common sensitizing allergens, frequent