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RESEARCH ARTICLE

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Responses of $CD27^{+}CD38^{+}$ plasmablasts, and $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ regulatory B cells during primary dengue virus 2 infection

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Abstract

Background: Humoral immunity is thought to play a central role in mediating the immunopathogenesis of dengue virus (DENV) infection; however, the B-cell responses elicited by primary DENV2 infection are incompletely understood. Follicular helper T cells (Tfh) are important to promote B-cell activation and differentiation.

Methods: The present study analyzed the detailed dynamic changes of circulating B-cell subsets and Tfh (cTfh) using flow cytometry to explore their responses to DENV2 infection.

Results: Thirty-six patients with DENV2 and 21 healthy individuals were included. The results showed that $CD27^{+}CD38^{+}$ plasmablasts emerged after DENV2 infection, and correlated with $CXCR5^{+}PD\text{-}1^{+}$ or $CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ cTfh, which increased after DENV2 infection, and correlated with DENV2 RNA viral loads. Significantly low levels of $CD27^{-}$ naïve B cells, and $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ regulatory B cells (Breg) were observed after DENV2 infection, which correlated negatively with $CXCR5^{+}PD\text{-}1^{+}$ or $CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ cTfh cells.

Conclusion: Overall, these results provide insights into the DENV2-elicited B-cell response and revealed previously unidentified $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg responses to DENV2 infection.

KEYWORDS
B-cell subsets, dengue fever, flow cytometry, follicular helper T cells

1 | INTRODUCTION

Dengue fever, a highly infectious disease transmitted by Aedes mosquitoes, is caused by four serotypes of dengue virus (DENV), which has led to 400 million infections annually in over 100 countries.[1] Most infections are subclinical; however, in some cases, DENV infection results in a variety of clinical symptoms, ranging from mild fever (dengue fever) to dengue shock syndrome and dengue hemorrhagic fever, which is fatal.[2,3] Currently, no approved antiviral drugs are available to treat DENV infection; therefore, the only management option for patients is symptomatic treatment.[4,5] DENV infection induces the expansion of memory B cells and plasmablasts.[6-10] Plasmablasts further differentiate into antibody-secreting plasma cells associated with an antigen-specific B-cell

immune response. This immune response plays an essential role in mediating the immunopathogenesis of acute DENV infection.[11-16] $CD24^{hi}CD27^{hi}$ B cells and $CD24^{hi}CD38^{hi}$ B cells have been reported as regulatory B cells (Breg), which decrease inflammatory reactions and suppress pro-inflammatory Th1/Th17 responses by producing interleukin (IL)-10 and/or transforming growth factor beta (TGF-β).[17] Siewe et al.[18] found that high IL-10-producing $CD24^{hi}CD27^{hi}$ Breg were related positively to the HIV viral load in patients with HIV infection. However, limited data are available on the dynamics of B-cell differentiation during DENV2 infection.

Follicular helper T cells (Tfh), situated in germinal centers (GCs), are a recently discovered $CD4^+$ T-cell subset that are vital for forming GCs and the humoral response.[19] Tfh are characterized by the expression of chemokine (C-X-C motif) receptor 5 (CXCR5), and the expression of other surface molecules, including programmed cell death 1 (PD-1) and inducible costimulatory (ICOS) represent different functional subtypes of Tfh.[20,21] Interestingly, circulating Tfh (cTfh) are found in the peripheral blood and have similar B-cell auxiliary function as GCs Tfh; however, they are more accessible for clinical detection.[22,23] Accumulating evidence has demonstrated that Tfh are likely to have a protective role in viral infection.[24] Herati et al.[11] and Koutsakos et al.[12] found that cTfh enhance effective B-cell immunity and helped to develop an influenza vaccine. Locci et al.[13] showed that cTfh correlated with HIV-specific antibodies. Haltaufderhyde et al.[14] observed that cTfh help the production of plasmablasts and DENV-specific IgM and IgG antibodies in patients with DENV infection. Achour et al.[25] revealed that the maturation of Tfh was controlled by Breg, and that Breg can inhibit the secretion of antibodies, mediated by Tfh.

Limited data are available on the DENV2-mediated immune response elicited by primary DENV2 infection. Therefore, the present study aimed to perform a longitudinal analysis of the continuum of B-cell subsets and cTfh following primary DENV2 infection.

2 | MATERIALS AND METHODS

2.1 | Subjects

We recruited 36 adult patients diagnosed as DENV2 infection and 21 healthy individuals (HIs) from Zhejiang Provincial People's Hospital. Serological screening was performed to rule out other bacterial and viral infections. EDTA-K_2 anticoagulated peripheral blood samples were collected at the time of diagnosis (D0) and at 15 days (D15) after diagnosis and detected within 24 hours. According to their clinical manifestations, patients were divided into three groups, including liver injury (−/+), kidney injury (−/+), and platelet normal/abnormal. The characteristics of the patients with DENV2 and HIs are listed in Table 1.

2.2 | Flow cytometry analysis

B-cell subsets and cTfh were characterized using following surface markers: CD19-FITC (Fluorescein isothiocyanate; Clone J4.119, Beckman Coulter, Indianapolis, IN, USA), CD38-APC (Allophycocyanin; Clone HB-7, BD Biosciences, San Jose, CA, USA), CD24-PerCP-Cy5.5 (Peridinin chlorophyll protein complex-cyanine 5.5; Clone ML5, BD Biosciences), CD27-PE (phycoerythrin; Clone L128, BD Biosciences), CD4-PC7 (PE-Cyanine7; Clone 13B8.2, Beckman Coulter), CXCR5- Alexa Fluor®488 (Clone RF8B2, BD Biosciences), PD-1-PerCP-Cy5.5 (Clone EH12.1, BD Biosciences), and ICOS-APC (Clone ISA-3, BD Biosciences). The gating strategy of B-cell subsets is shown in Supplementary Figure 1, while that of cTfh is shown in Supplementary Figure 2.

TABLE 1 Clinicopathological characteristics of the two groups of patients

Characteristic	Patients with DENV2	HIs	*p*
Sex, n	36	21	-
Male	19	11	-
Female	17	10	-
Age	48.5 ± 31.5	46.5 ± 13.5	0.8096
WBC (/μl)	3.88 ± 2.79	6.75 ± 2.69	<0.001
LYM (/μl)	1.64 ± 1.40	2.15 ± 1.05	0.046
PLT (*10^3/μl)	174.0 ± 146.2	194.16 ± 86.7	0.035
≥ 146	14	-	-
< 146	22	-	-
HCT (%)	32.56 ± 2.04	30.5 ± 1.79	0.0417
LDH (U/L)	281.57 ± 104.48	165.52 ± 40.17	<0.001
109–244	12	21	-
≥ 245	24	0	-
CK (U/L)	206.06 ± 55.04	71.45 ± 21.5	<0.001
< 250	30	21	-
≥ 250	7	0	-
Urea (mmol/L)	4.67 ± 2.28	4.54 ± 1.98	0.459
< 7.5	27	21	-
≥ 7.5	9	0	-
Urine protein			
Negative	27	21	-
Positive	9		-
Cr (μmol/L)	84.38 ± 50.72	72.5 ± 22.7	0.0414
< 123	27	21	-
≥ 123	9	0	-
ALT (U/L)	123.5 ± 107.5	23.5 ± 8.42	<0.001
< 40	25	21	-
≥ 40	11	0	-
AST (U/L)	198.5 ± 175.5	24.0 ± 5.62	<0.001
< 35	25	21	-
≥ 35	11	0	-

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CK, creatine kinase; Cr, creatine; HIs, healthy individuals; HTC, hematocrit; LDH, lactate dehydrogenase; LYM, lymphocyte; PLT, platelet; WBC, white blood cell.

2.3 | DENV2 RNA qualification

Reverse transcription PCR (RT-PCR) was used to confirm DENV2 infection. According to manufacturer's protocol (Invitrogen, Waltham, MA, USA), the Trizol LS reagent was used to obtain total cellular RNA from 250 μl of peripheral whole blood from patients with DENV2. A TaqMan one-step RT-PCR reaction that could detect 200 ng of RNA was used to evaluate DENV viremia. RT-PCR cycle threshold values (Ct) were used to describe the peripheral whole blood DENV RNA viral load. 100 copies/ml was the detection limit of this assay.

2.4 | Statistical Analysis

GraphPad Prism 5.01 software (GraphPad Inc., La Jolla, CA, USA) was used to analyze the data. Statistical tests included Spearman's *r* correlation and one-way analysis of variance (ANOVA) tests. The *p*-values <0.05 were regarded to indicate a statistically significant difference.

3 | RESULTS

3.1 | High levels of $CD27^+CD38^+$ plasmablasts, but low levels of $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg, occur during acute DENV2 infection

$CD27^+CD38^+$ plasmablasts represent a small subset of the memory B-cell pool and are at the differentiation stage of memory B cells and plasma cells. Our data showed that the frequency of $CD19^+$ B cells increased in patients with DENV2 infection while their number decreased compared with that in HIs (Figure 1A). A decrease in $CD27^-$ naïve B cells, and an increase in $CD27^+$ memory B cells (Figure 1C, 1E) and $CD27^+CD38^+$ plasmablasts, were observed at D0 and D15 in patients with DENV2 infection compared with those in HIs (Figure 1J), suggesting that naïve B cells might differentiate into memory B cells and plasmablasts during acute DENV2 infection, which can function rapidly in virus resistance.

Interestingly, we found low levels of $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg at D0 in patients with DENV2 infection; however, the numbers of these B cells were likely to recover by D15 (Figure 1F, 1H). These results demonstrated low levels of Breg during acute DENV2 infection.

3.2 | Expansion of cTfh in in patients with DENV2 infection

Tfh can help B-cell activation and differentiation; therefore, we further investigated the dynamic changes of cTfh. Compared with that in HIs, the frequency of circulating $CXCR5^+$ cTfh increased at D0 and then decreased at D15 while their overall number decreased (Figure 2A). We next analyzed different phenotypic cTfh cell subsets, based on the surface molecules CXCR5, ICOS, and PD-1. We found a higher frequency of $CXCR5^+PD\text{-}1^+$ cTfh at D0 and D15 (Figure 2E) and a higher frequency of $CXCR5^+ICOS^+$ and $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh at D0 in patients with DENV2 infection compared with those in HIs (Figure 2C, 2G). The frequencies of $CXCR5^+ICOS^+$ and $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh then decreased at D15 (Figure 2C, 2G). These results showed that the different phenotypic cTfh increased significantly during acute DENV2 infection and decreased gradually during early convalescence.

3.3 | The correlation between B-cell subsets and cTfh

Next, we further analyzed the association between B-cell subsets and cTfh. The correlation analysis demonstrated that $CD27^+CD38^+$ plasmablasts correlated positively with $CXCR5^+PD\text{-}1^+$ (Figure 3O) and $CXCR5^+IOCS^+PD\text{-}1^+$ cTfh (Figure 3T). We observed a negative correlation between $CD27^-$ naïve B cells and $CXCR5^+PD\text{-}1^+$ or $CXCR5^+IOCS^+PD\text{-}1^+$ cTfh (Figure 3K, 3P), also $CD24^{hi}CD27^{hi}$ or $CD24^{hi}CD38^h$ Breg and $CXCR5^+PD\text{-}1^+$ cTfh (Figure 3M, 3N). In addition, $CD24^{hi}CD27^{hi}$ Breg correlated negatively with $CXCR5^+IOCS^+PD\text{-}1^+$ cTfh (Figure 3R). However, other B-cell subsets did not correlate with cTfh (Figure 3A-3J, 3L, 3Q, 3S). These results suggested that cTfh promote the differentiation of naïve B cells into $CD27^+CD38^+$ plasmablasts instead of $CD24^{hi}CD27^{hi}$ or $CD24^{hi}CD38^{hi}$ Breg.

We further investigated the association between DENV-specific IgM antibodies and cTfh or B-cell subsets; however, no correlations were observed (data not shown).

3.4 | Positive correlation of $CXCR5^+ICOS^+$ or $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh with DENV2 RNA viral load in patients with DENV2 infection

Memory B cells, $CD27^+CD38^+$ plasmablasts, and cTfh were amplified in patients with DENV2 infection; therefore, we wondered whether these cells were associated with DENV2 RNA viral load at D0. To explore this, we measured DENV2 RNA and found a positive correlation between DENV2 RNA viral load and the frequency of $CXCR5^+ICOS^+$ or $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh at D0 (Figure 4B, 4D). However, no significant correlation was observed between the DENV2 RNA viral load and other cTfh (Figure 4A, 4C) or B-cell subsets (Figure S3).

3.5 | Low levels of B cells in patients with DENV2 with liver injury and platelet abnormality and high levels of cTfh in patients with DENV2 with kidney injury

Some patients with DENV2 infection develop serious complications, including kidney injury, liver injury, and platelet abnormalities;

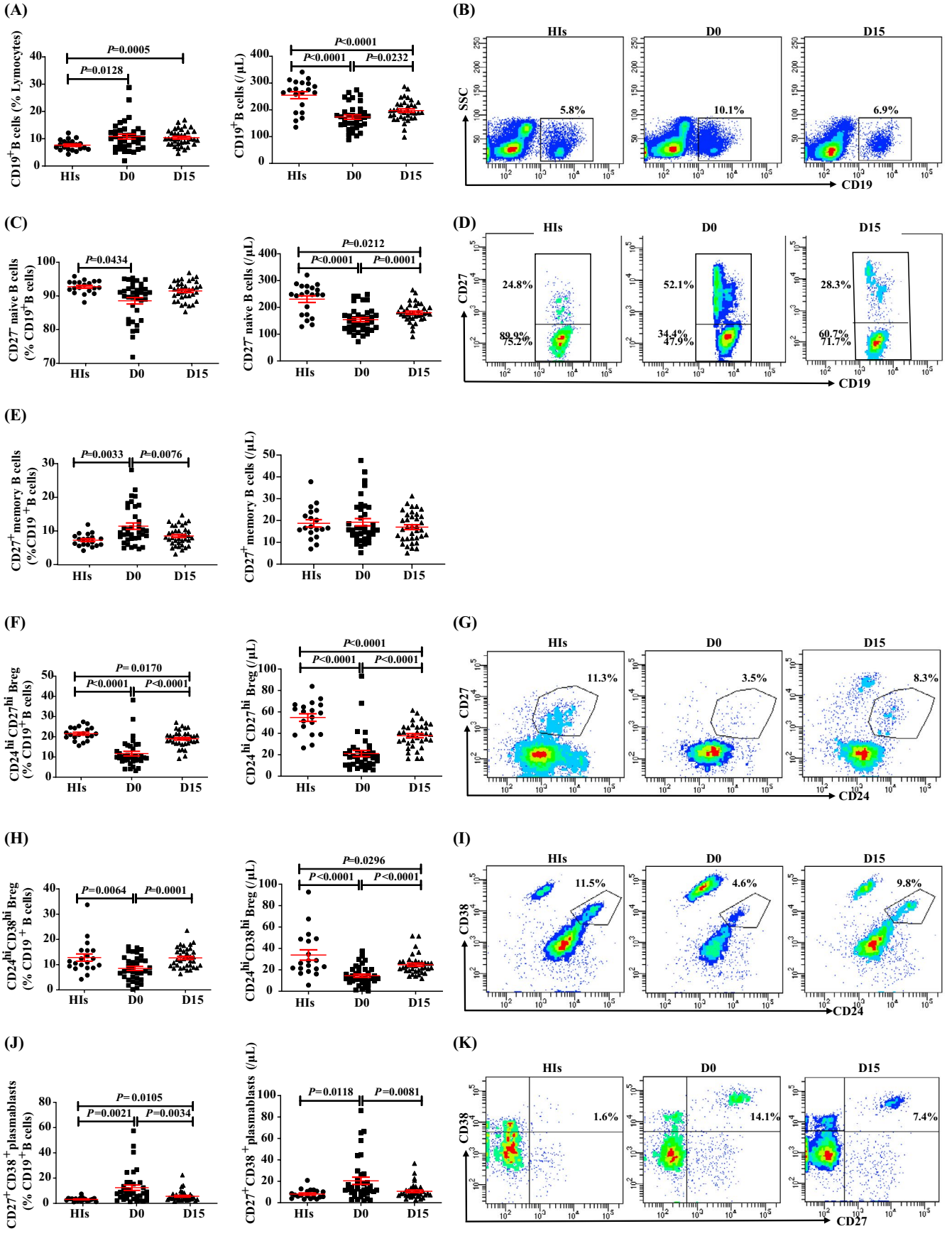
(A)
CD19+B cells (% Lymocytes)
P=0.0005
P=0.0128
HIs D0 D15
CD19+B cells (/μL)
P<0.0001
P<0.0001 P=0.0232
HIs D0 D15
(B)
HIs D0 D15
SSC
5.8%
10.1%
6.9%
CD19
(C)
CD27− naive B cells (% CD19+B cells)
P=0.0434
HIs D0 D15
CD27− naive B cells (/μL)
P=0.0212
P<0.0001 P=0.0001
HIs D0 D15
(D)
HIs D0 D15
CD27
24.8%
89.9%
75.2%
52.1%
34.4%
47.9%
28.3%
60.7%
71.7%
CD19
(E)
CD27+memory B cells (%CD19+B cells)
P=0.0033 P=0.0076
HIs D0 D15
CD27+memory B cells (/μL)
HIs D0 D15
(F)
CD24hiCD27hi Breg (% CD19+B cells)
P=0.0170
P<0.0001 P<0.0001
HIs D0 D15
CD24hiCD27hi Breg (/μL)
P<0.0001
P<0.0001 P<0.0001
HIs D0 D15
(G)
HIs D0 D15
CD27
11.3%
3.5%
8.3%
CD24
(H)
CD24hiCD38hi Breg (% CD19+B cells)
P=0.0064 P=0.0001
HIs D0 D15
CD24hiCD38hi Breg (/μL)
P=0.0296
P<0.0001 P<0.0001
HIs D0 D15
(I)
HIs D0 D15
CD38
11.5%
4.6%
9.8%
CD24
(J)
CD27+CD38+plasmablasts (% CD19+B cells)
P=0.0105
P=0.0021 P=0.0034
HIs D0 D15
CD27+CD38+plasmablasts (/μL)
P=0.0118 P=0.0081
HIs D0 D15
(K)
HIs D0 D15
CD38
1.6%
14.1%
7.4%
CD27

FIGURE 1 High levels of $CD27^+CD38^+$ plasmablasts and low levels of $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg during acute DENV2 infection. A. Frequency of lymphocytes (left) and the number (right) of $CD19^+$ B cells in HIs ($n = 21$) and patients with DENV2 infection ($n = 36$) at D0 and D15. Frequency of $CD19^+$ B cells (left) and the number (right) of $CD27^-$ naïve B cells (C), $CD27^+$ memory B cells (E), $CD24^{hi}CD27^{hi}$ Breg (F), $CD24^{hi}CD38^{hi}$ Breg (H), and $CD27^+CD38^+$ plasmablasts (J). Representative flow cytometry dot plots of $CD19^+$ B cells (B), $CD27^-$ naïve B cells (D), $CD27^+$ memory B cells (D), $CD24^{hi}CD27^{hi}$ Breg (G), $CD24^{hi}CD38^{hi}$ Breg (I), and $CD27^+CD38^+$ plasmablasts (K). The horizontal line represents the median for all data points, and the bar indicates the interquartile range

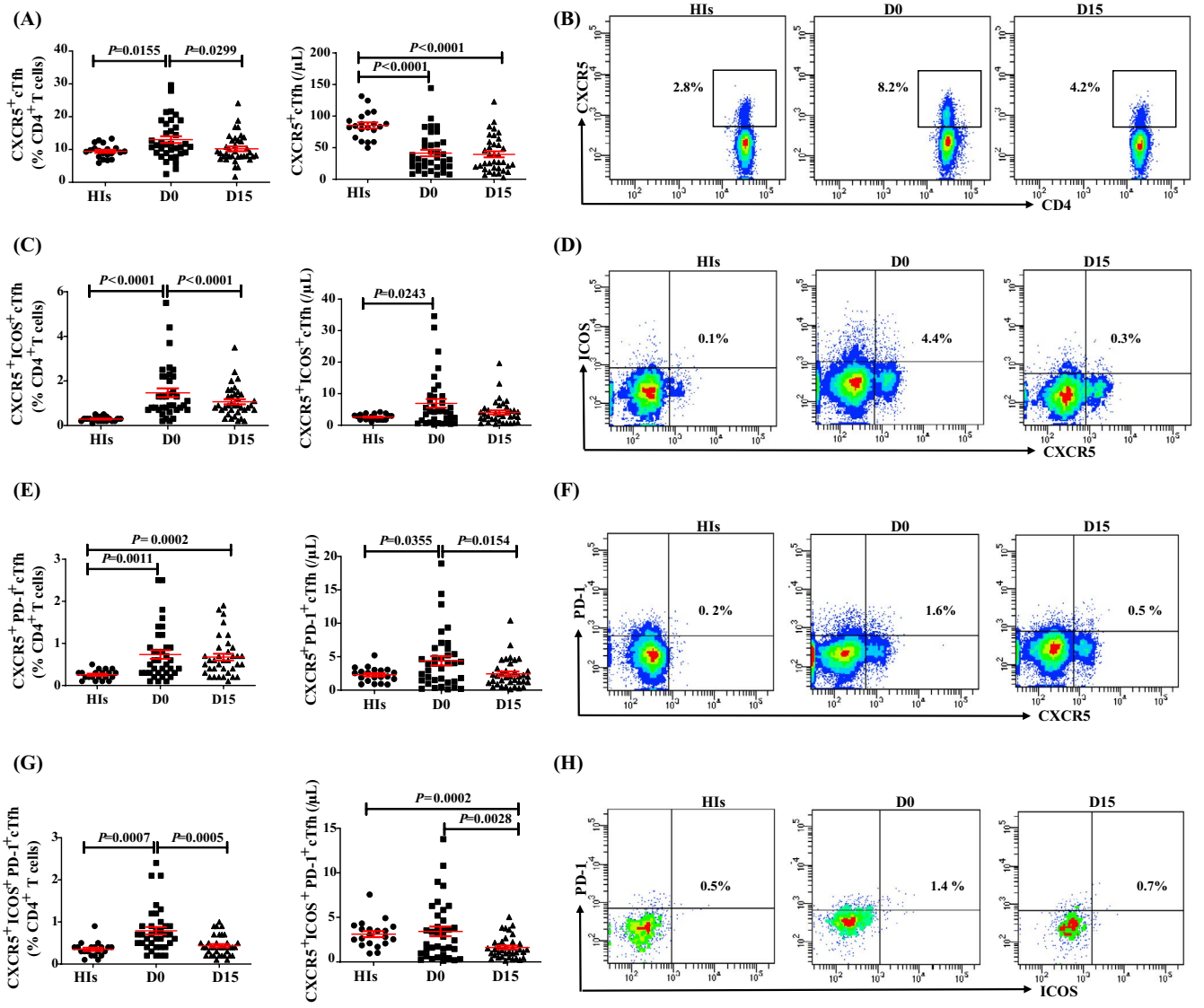

FIGURE 2 Expansion of cTfh in patients with DENV2 infection. Frequency of $CD4^+$ T cells (left) and the number (right) of $CXCR5^+$ (A), $CXCR5^+ICOS^+$ (C), $CXCR5^+PD\text{-}1^+$ (E), and $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh (G) in HIs ($n = 21$) and patients with DENV2 infection ($n = 36$) at D0 and D15. Representative flow cytometry dot plots of $CXCR5^+$ (B), $CXCR5^+ICOS^+$ (D), $CXCR5^+PD\text{-}1^+$ (F), and $CXCR5^+ICOS^+$ $PD\text{-}1^+$ cTfh (H) on $CD4^+$ T cells. The horizontal line represents the median for all data points, and the bar indicate the interquartile range

therefore, we investigated whether B-cell subsets or cTfh were associated with the complication. Data analysis showed that the number of $CD27^-$ naïve B cells and $CD27^+$ memory B cells decreased significantly in patients with liver injury and platelet abnormalities (Figure 5A, 5B). Interestingly, the number of $CXCR5^+$ cTfh and the frequency of $CXCR5^+PD\text{-}1^+$ or $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh were significantly higher in patients with DENV2 with kidney injury (Figure 5C). However, there was no difference in the numbers of cTfh in patients with DENV2 with liver injury (−/+) or platelet normal/abnormal and no difference in B-cell subsets in patients with DENV2 with kidney injury (−/+) (Figure S4).

4 | DISCUSSION

A longitudinal analysis of the B-cell subset response to primary DENV infection was provided in this study. We observed increased levels of $CD27^+$ memory B and $CD27^+CD38^+$ plasmablasts, but

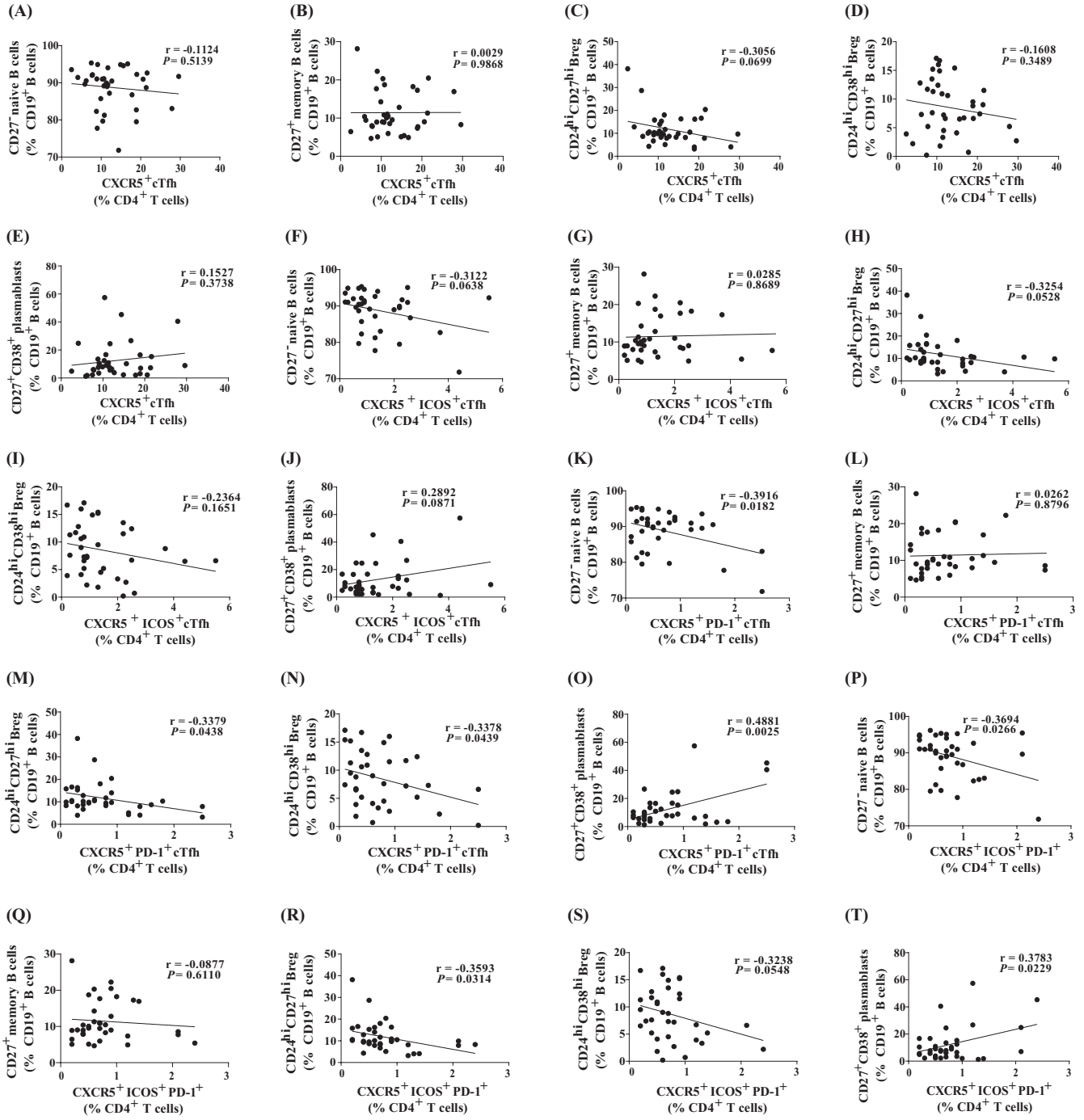

FIGURE 3 The correlation between B-cell subsets and cTfh. Correlation between the frequency of $CXCR5^+$ cTfh and $CD27^-$ naïve B cells (A), $CD27^+$ memory B cells (B), $CD24^{hi}CD27^{hi}$ Breg (C), $CD24^{hi}CD38^{hi}$ Breg (D), or $CD27^+CD38^+$ plasmablasts (E). Correlation between the frequency of $CXCR5^+ICOS^+$ cTfh and $CD27^-$ naïve B cells (F), $CD27^+$ memory B cells (G), $CD24^{hi}CD27^{hi}$ Breg (H), $CD24^{hi}CD38^{hi}$ Breg (I), or $CD27^+CD38^+$ plasmablasts (J). Correlation between the frequency of $CXCR5^+PD\text{-}1^+$ cTfh and $CD27^-$ naïve B cells (K), $CD27^+$ memory B cells (L), $CD24^{hi}CD27^{hi}$ Breg (M), $CD24^{hi}CD38^{hi}$ Breg (N), or $CD27^+CD38^+$ plasmablasts (O). Correlation between the frequency of $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh and $CD27^-$ naïve B cells (P), $CD27^+$ memory B cells (Q), $CD24^{hi}CD27^{hi}$ Breg (R), $CD24^{hi}CD38^{hi}$ Breg (S), or $CD27^+CD38^+$ plasmablasts (T)

decreased levels of $CD24^{hi}CD27^{hi}$ or $CD24^{hi}CD38^{hi}$ Breg, during acute DENV2 infection, which indicated that DENV2 infection induced B-cell activation and a high plasmablast response, which was consistent with previous studies.[9,10,26] At the early convalescence stage, increased levels of $CD27^+$ memory B cells and $CD27^+CD38^+$ plasmablasts were still observed, which indicated that memory B cells and plasmablasts are responsible for long-term humoral immunity.[6-8,27,28] B-cell activation is helpful to produce a substantial plasmablast response during acute DENV2 infection, resulting in high neutralizing antibody titers, which are

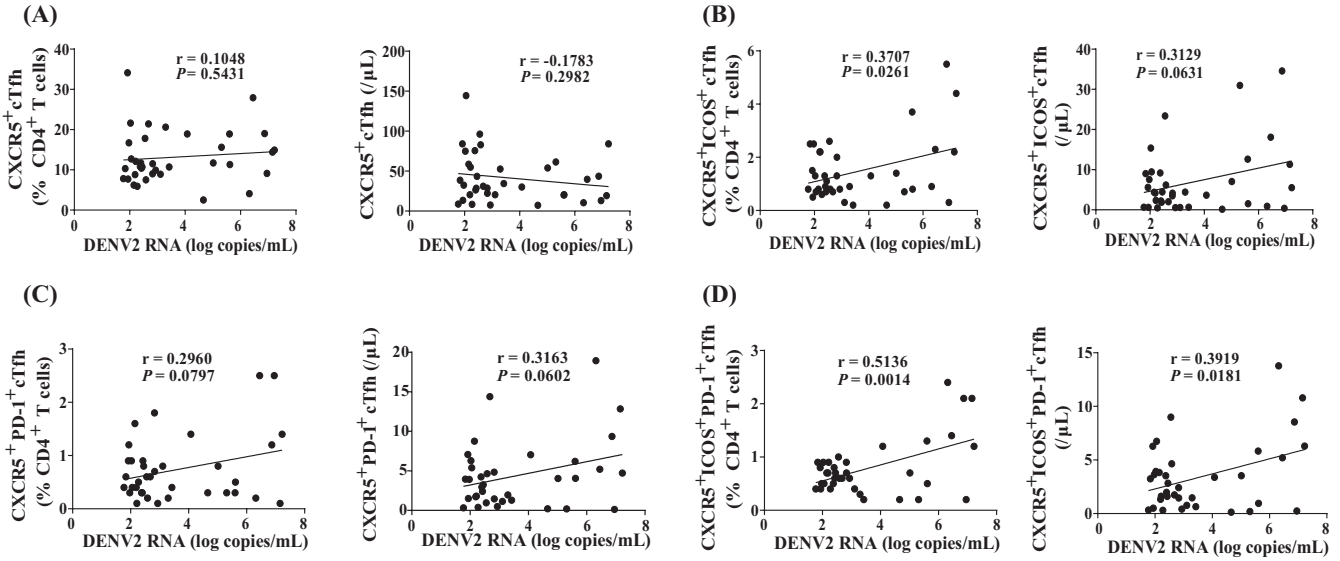

FIGURE 4 Positive correlation between $CXCR5^+ICOS^+$ or $CXCR5^+ICOS^+$ PD-1^+ cTfh with DENV2 RNA viral load in patients with DENV2 infection. Correlation between the frequency and number of $CXCR5^+$ (A), $CXCR5^+ICOS^+$ (B) $CXCR5^+PD\text{-}1^+$ (C), or $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh (D) and the DENV2 RNA viral load at D0

important for viral clearance and, thus, contribute to long-term protection from DENV infection Consequently, the large number of plasmablasts during acute DENV2 infection might be a direct response to DENV2-mediated activating signals. In addition, it has been reported that the plasmablast response can predict antibody titers in the early stage of recovery.[10,26]

The features of cTfh resemble those of Tfh in GCs, and analysis of cTfh is, thus, a suitable alternative strategy to investigating Tfh in GCs. The expression levels of PD-1 and ICOS are upregulated after activation, which improves the development of Tfh functions and promotes the migration of Tfh to GCs.[13,28] The most efficient promoters of GC B-cell antibody production are those Tfh that express both PD-1 and ICOS.[13] ICOS is related to the function of cTfh, and can promote the secretion of IL-21, the differentiation of cTfh, and the proliferation of B cells. PD-1 expressed on cTfh is related to the inhibition of T-cell receptor signaling, and in the absence of ICOS, PD-1 expression alone might regulate the activation of B cells.[29] We analyzed the dynamics of the different phenotypic cTfh and observed that $CXCR5^+ICOS^+$ and $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh were expanded significantly during acute DENV2 infection, and then gradually decreased at the early convalescence stage, which was similar to data reported in previous studies.[14,15] Most importantly, we found a positive correlation between $CXCR5^+ICOS^+$ or $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh and the DENV2 RNA viral load, which indicated that DENV2 infection induced cTfh-cell proliferation, and that cTfh promoted an increase in plasmablasts, which would result in the production of antibodies.

Furthermore, we demonstrated a positive correlation between $CD27^+CD38^+$ plasmablasts and $CXCR5^+PD\text{-}1^+$ or $CXCR5^+IOCS^+PD\text{-}1^+$ cTfh, suggesting that the most functional subsets of Tfh might generate regulatory signals that contribute to the differentiation of naïve B cells into plasmablasts during acute DENV2 infection. Most importantly, a negative correlation between $CD24^{hi}CD27^{hi}$ or $CD24^{hi}CD38^{hi}$ Breg and cTfh was identified. These results indicated that low levels of $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg might induce Tfh cell maturation and the Tfh cell-dependent control of humoral immunity, leading to DENV clearance.

Some patients with DENV2 infection develop serious clinical symptoms, such as kidney injury, liver injury, and platelet abnormalities. Interestingly, we found that the number of B cells decreased significantly in patients with DENV2 with liver injury and platelet abnormalities. This might have been caused by DENV2 infection inducing B cells to differentiate into plasmablasts, thereby secreting more antibodies, which eventually leads to liver damage and thrombocytopenia. Moreover, levels cTfh were higher in patients with DENV2 with kidney injury. Elevated Tfh induce naïve B cells to differentiate into memory B cells and plasmablasts, thereby secreting more antibodies. Antibodies form antigen-antibody complexes with their corresponding antigens, which might be deposited in the glomerular basement membrane, leading to kidney injury.

In conclusion, the temporal characteristics of the B-cell response to the early stage of primary DENV2 infection were defined in our study. They showed that high levels of plasmablasts were produced, accompanied by a decline of $CD24^{hi}CD27^{hi}$ and $CD24^{hi}CD38^{hi}$ Breg during acute DENV2 infection. Besides, the link between the B-cell response and cTfh demonstrated in the present study might contribute to the development of live attenuated DENV2 vaccines. Although this study was conducted on a small number of subjects, the results provide a better understanding of the humoral immunity during DENV2 infection. Further investigations are warranted to clarify the role of different Tfh cell subsets in inducing plasmablast production in patients with DENV2 infection.

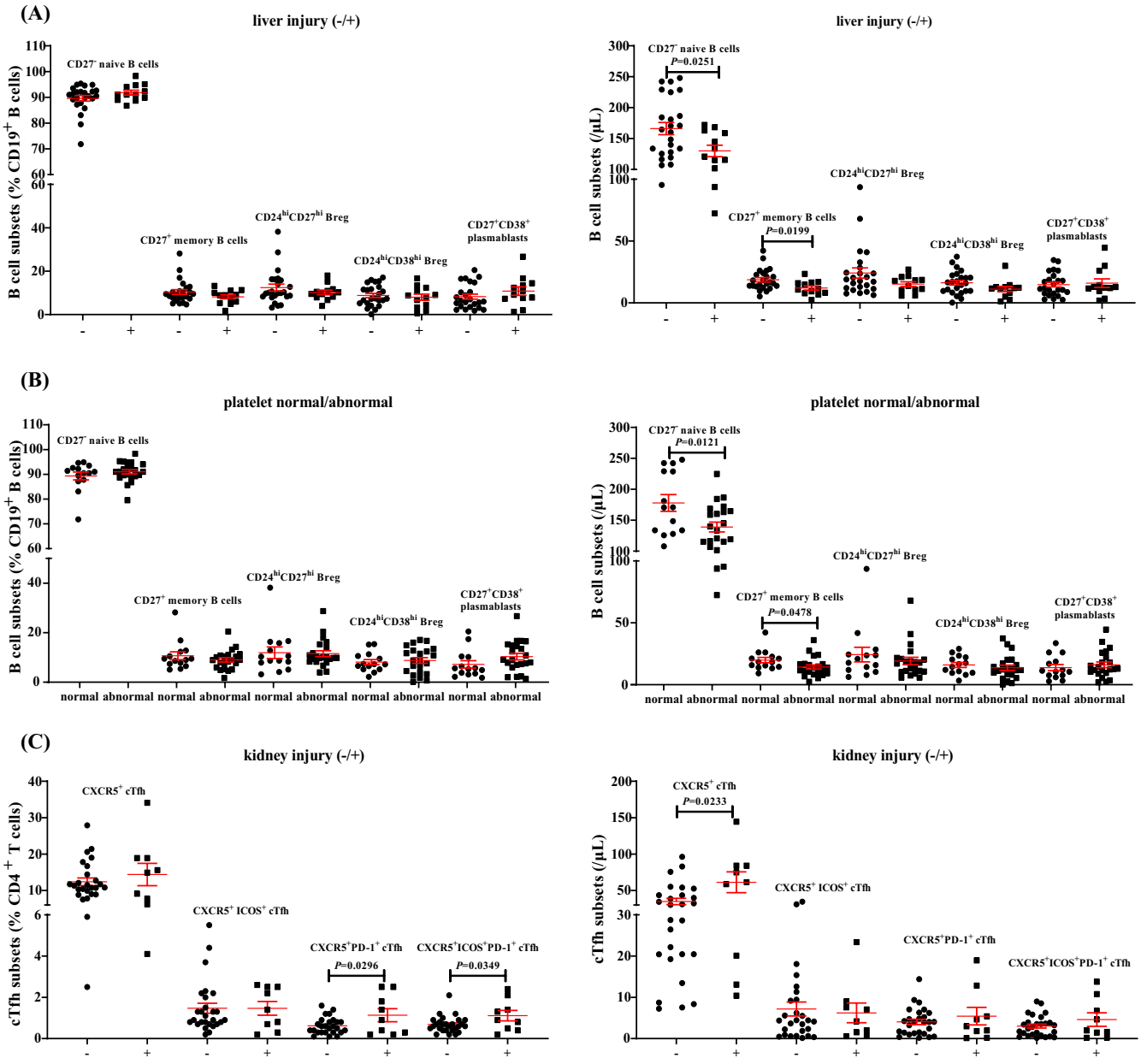

FIGURE 5 Low levels B cells in patients with DENV2 with liver injury and abnormal platelet counts, and high levels of cTfh in patients with DENV2 with kidney injury. Frequency of $CD19^+$ B cells (left) and number (right) of $CD27^-$ naïve B cells, $CD27^+$ memory B cells, $CD24^{hi}CD27^{hi}$ Breg, $CD24^{hi}CD38^{hi}$ Breg, and $CD27^+CD38^+$ plasmablasts in patients with DENV2 with liver injury (−/+) (A) and platelet normal/abnormal (B) at D0. Frequency of $CD4^+$ T cells (left) and the number (right) of $CXCR5^+$, $CXCR5^+ICOS^+$, $CXCR5^+PD\text{-}1^+$, and $CXCR5^+ICOS^+PD\text{-}1^+$ cTfh in patients with DENV2 with kidney injury (-/+) (C) at D0

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Liannv Qiu performed the research, collected and analyzed the data, and wrote the first draft. Chenshuang Lei performed the research. Hong Wang and Qinhua Yu collected and analyzed the data. JieJing Liu, Sufeng Chen and Zhao Zhao analyzed the data. All authors approved the final version of the article.

ETHICAL APPROVAL

This study was approved by the ethics committee of Zhejiang Provincial People's Hospital.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author Liannv Qiu on reasonable request.

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SUPPORTING INFORMATION

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