SARS-CoV-2-induced humoral immunity through B cell epitope analysis in COVID-19 infected individuals

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Abstract

The aim of this study is to understand adaptive immunity to SARS-CoV-2 through the analysis of B cell epitope and neutralizing activity in coronavirus disease 2019 (COVID-19) patients. We obtained serum from forty-three COVID-19 patients. Most individuals revealed neutralizing activity against SARS-CoV-2 assessed by a pseudotype virus-neutralizing assay. The antibody production against the spike glycoprotein (S protein) or receptor-binding domain (RBD) of SARS-CoV-2 was elevated, with large individual differences, as assessed by ELISA. We observed the correlation between neutralizing antibody titer and IgG, but not IgM, antibody titer of COVID-19 patients.

Background & Results

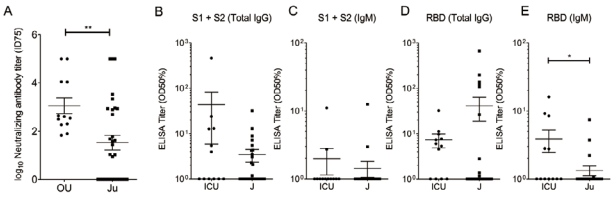
To understand the immune response to COVID-19, the analysis of virus-specific $CD4^+$ and $CD8^+$ T cells is required. $CD4^+$ T cell responses to S protein were robust and correlated with the magnitude of the anti-SARS-CoV-2 IgG and IgA titers.

Here, we addressed the humoral immune response by measuring antibody production against S protein and the neutralizing ability in convalescent patients from the intensive care unit of Osaka University Hospital (n = 12) and in Osaka City Juso Hospital (n = 31). The antibody production against S protein or RBD of SARS-CoV-2 was elevated, with large individual difference. In the analysis of the predicted the linear B cell epitopes, hot spots in the N-terminal domain of the S protein were observed in the serum from patients.

Significance of the research and Future perspective

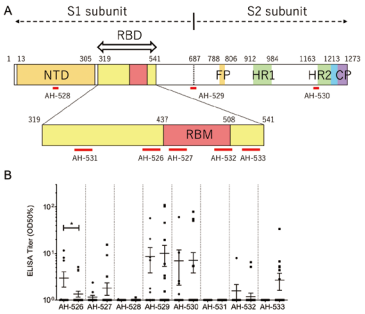
Osaka University Hospital primarily admits severe patients requiring the ICU, and patient status might be in the subacute phase. Juso Osaka City Hospital, in contrast, usually admits mild or moderate patients, and patient status might be in the convalescent phase. Interestingly, the average antibody titer to S protein was higher in samples from Osaka University Hospital, which was consistent with the high titers in severe patients. Of importance, several patients possessed neutralizing activity with a high titer of IgG for S protein, which may suggest the functional importance of IgG for S protein as neutralizing antibodies. In the B cell epitope analysis, the strongly binding B cell epitopes were located in the regions outside the RBD, such as the NTD, fusion peptide (FP) and heptad repeat (HR)2. Moreover, we evaluated the linear B cell epitope within nucleocapsid, membrane and envelope proteins, and most of the strongly binding B cell epitopes were located in nucleocapsid protein. In addition, the serum samples of non-COVID-19 patients collected in 2019 were hardly cross-reacted with nucleocapsid protein and were not cross-reacted with S protein and spike RBD protein.

Overall, the analysis of antibody production and B cell epitopes of the S protein from patient serum may provide a novel target for the vaccine development against SARS-CoV-2.

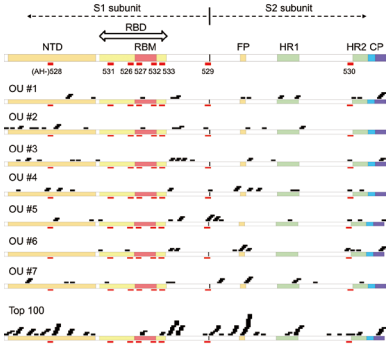


Neutralizing antibody titers and anti-SARS-CoV-2 IgG, IgM responses of COVID-19 patients

(A) The neutralizing antibody titers of serum antibodies against SARS-CoV-2 at an evaluation point of the 75% inhibitory dose (ID75).
(B,C) The serum titer against recombinant SARS-CoV-2 spike S1 + S2 protein. (B) Total IgG, (C) IgM, expressed as the OD at 450 nm and the half-maximal binding (OD 50%). (D,E) The serum titer against recombinant SARS-CoV-2 spike RBD protein. (D) Total IgG, (E) IgM, expressed as the OD at 450 nm and the OD 50%. OU, serum samples collected from patients in the ICU of Osaka University Hospital (n = 12); Ju, serum samples collected from patients in Osaka City Juso Hospital (n = 31).



Linear B cell epitopes on Spike protein of SARS-CoV-2 determined by ELISA. (A) Scheme of predicted linear B cell epitopes in the RBD or other regions of Spike protein in SARS-CoV-2. As shown in red bars, five regions were selected from the RBD, and three regions were selected from the NTD, S1 and S2 subunits. NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; CP, cytoplasm domain. (B) The serum titer against synthetic SARS-CoV-2 peptides (AH-526 to AH-533) is expressed as the half-maximal binding (OD 50%); closed square, serum samples collected from patients in Osaka City Juso Hospital (n = 31).



Mapping of the top 20 strongest binding peptide regions of serum samples. The scheme of lists and maps of the top 20 peptide regions with high intensity values for each individual (OU #1-#7). A series of 15-mer peptides overlapping by five amino acids (i.e., 1–15 aa., 5–20 aa., 10–25 aa., etc.) were displayed in this peptide array. The black block indicates the top 20 strongest binding peptide regions with serum samples from each individual and the top 100 strongest binding peptide regions for all samples. The red bars show the candidate linear B cell epitopes: five regions from the and three regions from the NTD, S1 and S2. NTD, N-terminal domain.

Patent

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Keyword SARS-CoV2, antibody, epitope