Neutralization of the emerging SARS-CoV-2 variant Lambda by antibodies elicited by inactivated virus and mRNA vaccines

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Brief Communication

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Abstract

Here, we used pseudotyped viruses to characterize the neutralization capacity of antibodies elicited by the CoronaVac and BNT162b2 vaccines against the emerging variant of interest Lambda. We observed that BNT162b2 elicits higher neutralizing antibody titers than CoronaVac, ranging from 5.8-fold for the ancestral spike to 9.4-fold for the Lambda variant. Neutralization against D614G, Alpha, Gamma, and Lambda variants was reduced between 1.78 to 3.05-fold for CoronaVac and 1.10 to 1.87-fold for BNT162b2. Structural analyses of the Lambda spike show significant changes in antigenic sites including the 246–252 deletion in an antigenic supersite at the NTD loop and, L452Q/F490S within the RBD that may account for immune escape. Our analysis of pseudotyped viruses also suggests increased infectivity driven by the Lambda spike. Together, our data indicate that inactivated virus and mRNA vaccines elicit different levels of neutralizing antibodies with different potency to neutralize SARS-CoV-2 variants, including the emergent variant Lambda.

Main

The emergence of SARS-CoV-2 variants of concern (VOC) and interest (VOI) has been a hallmark of the COVID-19 pandemic during 2021¹. Classification of VOC and VOI by the WHO is highly dynamic with emergent SARS-CoV-2 variants such as Lambda (lineage C.37) being recently recognized as VOI while others such as Epsilon (lineage B.1.427/29), Zeta (lineage P.2) and Theta (lineage P.3) being no longer considered as VOI². Variants of concern and interest are characterized by the presence of mutations located in key positions of the spike protein, including those in the receptor binding domain (RBD) such as N501Y (shared by VOC Alpha, Beta, and Gamma) associated with increased ACE2 binding and infectivity or K417N/T and E484K (shared by VOC Beta and Gamma) associated with escape from neutralizing antibodies¹. The spike protein of the emergent VOI Lambda has a unique pattern of seven mutations (G75V, T76I, Δ246–252, L452Q, F490S, D614G, T859N) not previously described in other VOC or VOI³.

The presence of mutations in antigenic sites characteristic of VOC and VOI has raised concerns regarding their impact on the neutralizing capacity of antibodies elicited by currently available vaccines, which were designed using the ancestral spike as a reference. Indeed, several studies have shown that VOC and VOI escape to neutralization from antibodies elicited by vaccines, although at different extents¹. Interestingly, recent studies suggested that neutralizing antibody titers elicited by vaccines should be considered as a correlate of protection^{4,5}. Under this scenario, the emergence of SARS-CoV-2 variants carrying mutations in antigenic sites within the spike protein and their impact on neutralization by antibodies elicited by vaccines requires continuous monitoring.

The virus inactivated vaccine CoronaVac and the mRNA vaccine BNT162b2 were identified amongst those with the lower and the higher correlates of protection, respectively^{4,5}. Therefore, we sought to determine and compare the neutralization capacity of serum samples obtained from 133 healthcare

workers receiving the complete scheme of these vaccines during the Chilean Ministry of Health vaccination campaign (Supplementary Table 1).

We generated pseudotyped viruses carrying either the ancestral SARS-CoV-2 spike protein (wild type; lineage A), the spike protein carrying the D614G mutation (lineage B) as well as the spike from variants Alpha (lineage B.1.1.7), Gamma (lineage P.1) and Lambda (lineage C.37) as previously described⁶. Neutralization assays using the pseudotyped virus carrying the ancestral spike revealed that the geometric mean of the ID₅₀ titer was 138.7 (95% CI, 114.7 to 167.7) for the individuals receiving the inactivated virus vaccine, while it was 803.8 (95% CI, 652.9 to 989.6) for those inoculated with the mRNA vaccine (Fig. 1a, left and Supplementary Tables 2 and 3). These data indicate that the BNT162b2 vaccine elicits 5.8-fold higher neutralizing antibody titers against the ancestral spike protein compared to CoronaVac (Fig. 1a, left). Neutralization assays using pseudotypes carrying the D614G mutant or the Alpha, Gamma, and Lambda variants resulted in geometric mean ID₅₀ titers of 101.2 (95% CI, 82.30 to 124.4), 68.10 (95% CI, 52.81 to 87.82), 59.23 (95% CI, 46.03 to 76.21) and 45.48 (95% CI, 36.11 to 57.29), respectively, for the CoronaVac samples while they were 728.2 (95% CI, 595.0 to 891.2), 488.4 (95% CI, 389.9 to 611.7), 427.8 (95% CI, 340.8 to 536.9) and 427.7 (95% CI, 343.6 to 532.5) for the BNT162b2 samples (Fig. 1a, right and Supplementary Tables 2 and 3). Neutralizing antibody titers against spike variants were 7.2 to 9.4-fold higher for BNT162b2 vaccinees (Fig. 1a, right). Compared to the ancestral spike, neutralizing antibody titers elicited by CoronaVac decreased by a factor of 1.37 for the D614G mutant, 2.03 for the Alpha spike, 2.33 for the Gamma spike, and 3.05 for the Lambda spike (Fig. 1b, left and 1c, upper and Supplementary Tables 2 and 3). Neutralizing antibody titers elicited by BNT162b2 decreased by a factor of 1.10, 1.65, 1.88, and 1.87 for the D614G mutant or the Alpha, Gamma, and Lambda variants, respectively (Fig. 1b, right and 1c, bottom and Supplementary Tables 2 and 3).

Demographic analyses of our study cohort indicate no obvious correlation between sex, age, body-mass index (BMI), or smoke status and neutralizing antibody titers regardless of the vaccine platform they received (Supplementary Fig. 1a-d).

Analysis of immunogenic regions shows that the three tested SARS-CoV-2 variants display mutations in immunodominant epitopes of the receptor binding motif targeted by class I and class II antibodies (Fig. 2a and 2b). Whereas Alpha and Gamma carry a polar to aromatic change (N501Y) and Gamma changes the charge of hydrophilic amino acids (E484K, K417T) in the receptor binding motif (RBM) within the RBD, the Lambda variant shows hydrophobic to polar substitutions (L452Q, F490S) in the same region (Fig. 2a and 2b). The L452Q mutation is similar to the L452R mutation recently shown to confer both increased infectivity and escape from neutralizing antibodies^{7,8}. The F490S mutation in the Lambda RBD has also been associated with escape from neutralizing antibodies^{7,9}. On the other hand, all three variants show substitutions in the amino-terminal domain (NTD) antigenic supersite. However, while the Alpha variant carries a single amino acid deletion (Δ Y144) in the b-hairpin and Gamma has two substitutions in the N-terminus (L18F, T20N), the Lambda variant harbors a seven amino acid deletion of the supersite loop (Δ 246–252) recently described as an antigenic supersite¹⁰. Indeed, structure analyses

show that both L452Q/F490S and $\Delta 246-252$ reside in the interface between the spike protein and monoclonal antibodies targeting the RBD and the NTD, respectively (Supplementary Fig. 2). This distinctive substitution pattern within antigenic sites may, at least in part, explain the escape of the Lambda spike from CoronaVac and BNT162b2-induced polyvalent sera.

Considering the recent emergence of the Lambda variant together with its unique pattern of mutations in the spike protein together with its ability to escape from neutralizing antibodies elicited by both vaccines, we then used equivalent amounts of each pseudotyped viruses and compared the capacity of the respective spike protein to drive viral entry. Interestingly, we observed that infectivity of the pseudotype carrying the Lambda spike was higher than the D614G mutant and the Alpha and Gamma variants both at low and high viral titers (Supplementary Fig. 3). Although some RBD mutations such as N501Y and L452R have been linked to increased infectivity^{1,7,8}, mutations within intermediate folding domains may also change infectivity by affecting the spike molecular dynamics^{11,12}. In this sense, the spike protein of the Lambda variant displays the substitution T859N that lies in the neighborhood of G614 in the apposed protomer (Fig. 2c). In the ancestral spike protein, the D614-T859 interaction was proposed to modulate the stability of the inter-protomer interface, and earlier molecular dynamics simulations suggest that fluctuations in the native contacts may facilitate the transition between up and down states in the spike's RBD¹³. In fact, the G614 form increases the occupancy of the one-up state, which accounts for its enhanced infectivity¹⁴.

We also noticed that T859 forms hydrophobic contacts with F592 in the apposed protomer (Fig. 2c). Interestingly, conservation analysis amongst betacoronaviruses spikes revealed that when a phenylalanine is present at an equivalent 592 position, it is coupled to threonine at 859 (Supplementary Fig. 4). This phenylalanine-threonine conservation was observed exclusively in the sarbecovirus group, which includes SARS-CoV, PCoV (pangolin), RatG13 (bat) and SARS-CoV-2 (Supplementary Fig. 4). Considering the relevance of the T859 in the interprotomer interface interaction, the effects of the T859N mutation characteristic of the Lambda spike warrant further investigation.

Data presented here confirm that neutralizing antibody titers elicited by the mRNA vaccine BNT162b2 are higher than those elicited by the inactivated virus vaccine CoronaVac in a real-life vaccination setting. Previous studies have shown that most of the neutralizing antibodies are directed to antigenic sites present in the RBD and, to a lesser extent, in the NTD¹. In this sense, BNT162b2 delivers an mRNA sequence coding a spike protein stabilized in the prefusion conformation that keeps intact the RBD and NTD-containing S1 domain¹⁵. Conversely, studies performed on a b-propiolactone inactivated virus like that used in the CoronaVac vaccine showed that 74.4% of the spike proteins are in the post-fusion conformation thus, with a minimal fraction (25.6%) of the spike conserving the major antigenic sites present in the S1 domain¹⁶. These differences in the availability of antigenic sites may explain, at least in part, the differences in the neutralizing antibody titers observed between both vaccine platforms.

Interestingly, a nationwide study for BNT162b2 carried out in Israel revealed an effectiveness for the complete scheme of 97% in preventing symptomatic COVID-19, 97.5% against severe or critical disease,

and 96.7% against COVID-19-related deaths¹⁷. A similar study for CoronaVac carried out in Chile showed a 65.9% effectiveness of the complete scheme in preventing COVID-19, a 90.3% in preventing ICU admission, and an 86.3% in COVID-19-related deaths¹⁸. The differences observed in the neutralizing antibody titers elicited by these two vaccines and the ability of these antibodies to neutralize SARS-CoV-2 variants may help to explain the differences observed, especially in the effectiveness to prevent symptomatic disease (97% for BNT162b2 vs. 65.9% for CoronaVac) in a real-life vaccination setting involving millions of individuals. Therefore, our data provide further evidence favoring the use of neutralizing antibody titers as a correlate of protection, as recently suggested^{4,5,19}. The use of a correlate of protection is critical when analyzing the potentials of booster schemes in specific groups such as elderly or immunosuppressed individuals.

Data presented here also show for the first time that the unique pattern of mutations present in the spike protein of the emerging VOI Lambda confers escape to neutralizing antibodies elicited by CoronaVac and to a lesser extent by BNT162b2. This effect is even higher than observed for the VOC Alpha and Gamma for CoronaVac vaccinees. Since the 246–252 deletion in the NTD and the L452Q/F490S mutations in the RBD are located in antigenic sites, these mutations may probably reduce the affinity of neutralizing antibodies. Our data also suggest that spike mutations in the Lambda variant confer increased infectivity. In particular, the potential effects of the T859N deserve further investigation. These observations may contribute to explain the fast rise of this VOI in South American countries and may serve as an alarm to prevent its expansion to other continents.

Declarations

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AUTHORS CONTRIBUTIONS

MLA, CPC, AG, FVE, and RSR designed the study. MLA, LAP, and AB designed mutants and performed the experiments. AG and CPC provided clinical samples. MLA, FP, and FVE performed statistical analysis.

MMO, SP, and MAN performed structural and bioinformatic analyses. FVE and RSR wrote the manuscript. FVE, AG, and RSR acquired funding. All authors approved the final version of the manuscript.

ROLE OF THE FUNDING SOURCE

The funding source had no role in the design of this study or its execution, analyses, interpretation of the data, or decision to submit the results.

DECLARATION OF INTERESTS

All authors declare no conflicts of interest

DATA AVAILABILITY

Data and reagents used in this work will be available upon reasonable request to the corresponding authors.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine at Universidad de Chile (Projects N° 0361-2021 and N° 096-2020) and Clínica Santa Maria (Project N°132604-21). All donors signed an informed consent, and their samples were anonymized. (Statement also provided in the supp files, FYI.)

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Figures

for each pseudotyped virus. Factor changes are shown as the difference of the geometric mean titer in the ID50 as compared with that of the Wild type pseudotyped virus. Statistical analyses were performed with the two-tailed Kruskal–Wallis test after adjustment for the false discovery rate. (C) Results are shown as the difference in neutralization titers of matched samples. P values for the comparison of the ID50 were calculated with the Wilcoxon signed-rank test.

Figure 2

Structural analysis and location of the mutations in different variants. a) Schematic representation of the different domains containing spike mutations. Color codes according to the variant correspond to that of Figure 1. b) Mapping of mutation sites on the spike structure (PDBid: 7BNN). c) Insight on the neighborhood of T859 at the interface of two spike protomers. The inset on the right shows the hydrophobic interaction with F592 in the next protomer. G164 is also shown for reference.

Supplementary Files

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