The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity


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BIOL 368: Bioinformatics Laboratory
November 19, 2020
Previous studies have observed that the SARS-CoV-2 spike protein is mutating and is highly glycosylated. 106 spike protein mutations were selected to be analyzed for infectivity and antigenicity. D614G mutations, alone or in combination with other amino acid changes, are significantly more infectious. Deletion of both N331 and N343 glycosylation drastically reduced viral infectivity. Mutations such as N234Q, L452R, and V483A were markedly resistant to neutralizing antibodies.
The SARS-CoV-2 spike protein has been mutating and is highly glycosylated

- Because SARS-CoV-2 enters target cells via the ACE2 receptor, the spike protein determines viral infectivity and transmissibility.
- Changes in the surface protein amino acids can impact viral function and interaction with the host's neutralizing antibodies.
- A previous study suggested that the D614G mutation could increase the fatality rate by altering S protein conformation and increasing infectivity.
- To gain a better understanding of natural variants, 106 S protein variants were analyzed for infectivity and reactivity to neutralizing antibodies.
Fig. 1

- A: Variants and combined variants with D614G across the entire S gene excluding the RBD region.

- B: Variants in RBD.

- C: Mutants at the putative glycosylation sites (22 sites). This group includes mutations introduced at all 22 putative glycosylation sites (N to Q), a combination of two glycosylation site mutations in RBD, and three naturally occurring variants, N74K, N149H, and T719A, with ablated glycosylation sites.

- High-frequency amino acid change sites (frequency > 0.1%) are highlighted in red. See also Table S1.
A total of 26 different cell lines were infected with pseudotyped viruses with either SARS-CoV-2 S (A) or VSV G (B).

The infected cell lysates were diluted by 10× and analyzed for luminescence activities (RLU).

All results were obtained from three independent experiments (mean ± SEM).

When the RLU for the tested cell reached 1% of that for Huh-7, it was deemed as a permissive cell line.

The dash line indicates the 1% RLU value of Huh-7 cell.
106 pseudotyped viruses show differing infectivity rates in the 293T-hACE2 cell line

- 24 strains were deemed low-infectivity, with RLU reading decreased by 4- to 100-fold, compared to the reference strain.
- Variant V341I and the investigative N331Q+N343Q glycosylation mutant showed no-infectivity, with over 100-fold decreases compared to the reference strain.
D614G mutations show significant increases in infectivity

- In all four cell lines, D614G variants showed increased infectivity compared to the reference strain.
- No difference was noted between single or combined variants, further indicating that D614G is responsible for the observed increase.
Differential Sensitivity of the Natural Variants and Experimental Mutants to a Panel of Convalescent Serum Samples

- Increase in neutralization activity in N165Q, N149H, N149Q, N1173Q, N709Q, N354D
- Major decrease in neutralization activity in H519P
- Decrease in neutralization activity in F490L
Summary of the data from ten serum samples, with the values presented as mean ± SEM. The horizontal dashed lines indicate the threshold of 4-fold difference. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001

- F338L, V367F, I468F, I468T, and V615L were even more sensitive to the convalescent sera compared with reference strain.
- More variants were found to be resistant to the convalescent sera including single and double amino acid change.
mAb Epitopes and Antigenicity Related Sites

- A475V, L452R became resistant to some neutralizing antibodies
- N165Q became more sensitive
- N234Q was markedly resistant to neutralizing antibodies.
- F490L became resistant to some neutralizing antibodies
N165Q and N234Q were identified to be important binding sites for glycosylation

- A475V, N234Q, and F490L modifications all displayed less antibody effectiveness
- N165Q, I468T, I468F, V367F modifications all displayed increased antibody effectiveness
N165Q, V367F, A475V, and N234Q produced consistent results

- Sequences that had significant data did not vary substantially based on antibody.
- Sequences without significant data remained insignificant
N165Q H519P and F490L produced consistent results

- Both H519 and F490L were significantly less affected by antibodies
- Substantial variation among sequences that were affected more by antibodies
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<tr>
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<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tr>
<td><strong>Number of variants or mutants</strong></td>
<td>29</td>
<td>51</td>
<td>26</td>
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<tr>
<td><strong>Increased sensitivity to</strong></td>
<td>none</td>
<td>V367F, Q409E, Q414E, I468F, I468T, Y508H, A522V</td>
<td>N165Q, N709Q</td>
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<td><strong>neutralizing mAbs</strong></td>
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<td><strong>Decreased sensitivity to</strong></td>
<td>A831V</td>
<td>N439K, L452R, A475V, V483A, F490L, Y508H, D614G+A435S, D614G+I472V&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N234Q</td>
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<tr>
<td><strong>neutralizing mAbs</strong></td>
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<td><strong>Increased sensitivity to</strong></td>
<td>V615L</td>
<td>F338L, V367F, I468F, I468T</td>
<td>N149H, N149Q, N165Q, N331Q, N354D, N709Q, N1173Q</td>
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<td><strong>convalescent sera</strong></td>
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<sup>a</sup>D614G+I472V is the only variant with increased infectivity and decreased sensitivity to neutralizing mAb and convalescent sera. It is of note only one sequence is recorded in GISAID.
Variation in infectivity and antigenicity can be used in vaccine and therapeutic antibody development

- Through analyzing 106 S protein variants, it was found that D614G is a particularly significant change, and has been rapidly accumulating.
- Most of the natural variants that affected sensitivity to neutralizing mAbs were located in the RBD region.
- The 13 mAbs used in the study appear to have different inhibitory effects on the variants.
  - It would be interesting to study the effects of a therapeutic regimen comprised of two or more mAbs.
- Glycosylation sites affect infectivity and antigenicity, indicating that glycan could impact SARS-CoV-2 replication and vaccine-induced immune response.
Summary

- Of 106 S protein variants, D614G mutations, alone or in combination with other amino acid changes, showed significantly higher infectivity.
- Deletion of both N331 and N343 glycosylation drastically reduced viral infectivity.
- Mutations such as N234Q, L452R, and V483A were markedly resistant to neutralizing antibodies.
- Glycosylation sites affect infectivity and sensitivity to neutralizing antibodies, indicating that glycan could impact SARS-CoV-2 replication and vaccine-induced immune response.
References

Acknowledgements

We would like to thank Dr. Dahlquist, our TA Annika, and the rest of the Loyola Marymount University Biology Department for allowing us to fulfill this project and providing us with the resources to complete this project.