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Mutagenesis of the of SARS-Coronavirus-2 Spike Gene

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Abstract

Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, has become a global pandemic resulting in over 6 million deaths worldwide. The virus binds to host cells through its spike (S) attachment protein, composed of two subunits: S1 and S2. The S protein is a target for vaccine development, with neutralizing antibodies blocking ACE2 binding to prevent infection. However, SARS-CoV-2 continues to evolve and accumulate mutations in the S protein, including L452R, T478K, D614G, P681R, and D950N, which can increase infectivity and evade host immune functions. To study the functions of these mutations, we randomly combined them in the alpha variant S gene using primers of differing concentrations, generating a library of transformants with unique combinations of mutations. Sequencing to confirm mutations is pending, and the future goal of this project is to use safe-to-use virus-like particles psuedotyped with the mutated SARS-CoV-2 S proteins which will be used in entry and immune cell deregulation assays.

Introduction

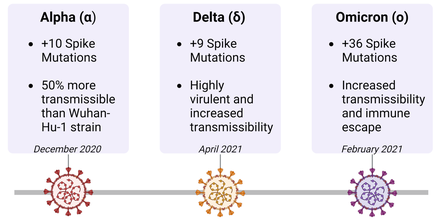
The COVID-19 pandemic caused by the SARS-CoV-2 virus has impacted millions of lives worldwide. One of the main challenges in controlling the pandemic is the emergence of new variants with mutations in the spike protein, the primary target of vaccines and antibody therapies. The S protein of SARS-CoV-2 binds to the host receptor and mediates viral and host membrane fusion. It is composed of two subunits, S1 and S2. The S1 subunit houses the receptor binding domain (RBD) that is responsible for recognizing host receptor angiotensin-converting enzyme 2 (ACE2). The S2 subunit is responsible for viral fusion with host cell membrane. Previous studies have illustrated the consequences that arise from mutations to the S protein. For example, A hallmark mutation in the delta variant P681R located in a furin cleavage site separating the S1 and S2 subunits was shown to enhance viral transmission and infection by allowing furin to cleave the site more efficiently. Furin cleavage separates S1 and S2 subunits and is followed by a second cut by host TMPRSS2 which exposes the fusion spike. After exposing the fusion spike, it burrows into the host cell membrane and results in fusion of viral and cell membranes.[1,2] A furin site that is more efficiently cleaved results in enhanced viral entry.[3] Furthermore, the delta variant of SARS-CoV-2 contains 4 additional mutations that we are investigating, namely L452R, T478K, D614G, and D950N which are associated with increased transmissibility and disease severity.[4,5,6] These mutations are significant as they can be investigated using our assays for entry and immune cell deregulation. Recent research indicates a trend of emerging variants with improved fitness due to mutations in the S protein, highlighting the need to understand how these mutations alter viral functions. The aim of this study is to generate mutations in the SARS-CoV-2 alpha variant S attachment gene using SARS-CoV-2 delta variant S attachment gene mutations, to create a library of mutants that can be tested in entry and immune cell deregulation assays. This research will aid in the development of vaccines, diagnostics, therapeutics, and policies important for hospitals and public health.

Project Goals

- Generate specific mutants of the spike protein of SARS-CoV-2 virus
- Replace amino acids in the SARS-CoV-2 alpha variant S gene with the corresponding mutations in the SARS-CoV-2 delta variant S gene (L452R, T478K, D614G, P681R, D950N)
- Long-term goal:
  - Test if our library of mutants affect viral entry kinetics and immune cell activation

SARS-CoV-2 Timeline of Emerging Variants

- SARS-CoV-2 has evolved numerous times
- The emergence of COVID-19 variants underscores the necessity for research into viral mutations to better understand their potential impact on transmission dynamics, disease severity, and public health interventions
- With more research, better treatment options can be established



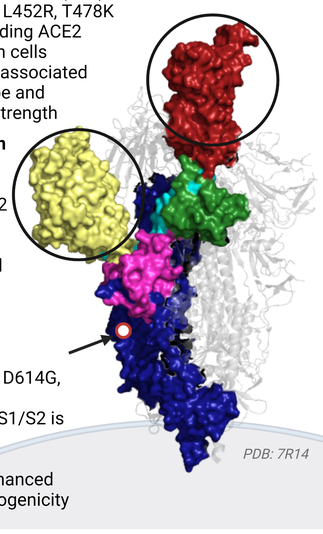
Structure and Function of SARS-CoV-2 Spike Protein

SARS-CoV-2 Spike Protein Structure

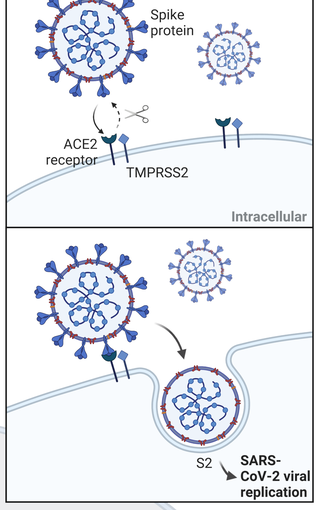
RBD: Receptor binding domain (Red)
- Includes mutations L452R, T478K
- Responsible for binding ACE2 receptors on human cells
- Mutations here are associated with immune escape and enhanced binding strength

NTD: N-terminal domain (Yellow)
- Stabilizes the RBD and regulates S1/S2 cleavage.
- Mutations in the NTD are associated with increased binding affinity of the RBD to ACE2.

S1/S2 (Blue)
- Includes mutations D614G, P681R
- Boundary at which S1/S2 is cleaved by furin
- Mutations here are associated with enhanced infectivity and pathogenicity

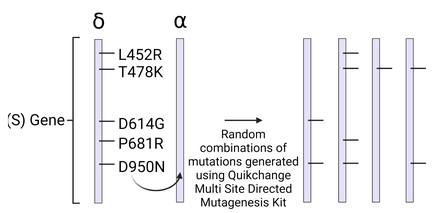


SARS-CoV-2 Entry through Host ACE2



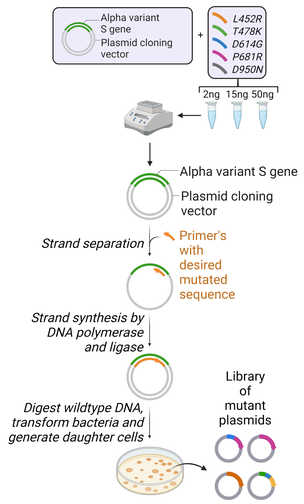
Strategy for Generating a Library of S Gene Mutants

- The goal was to create a library of plasmids containing random combinations of delta variant mutations
- Three mutagenesis reactions were conducted using different primer concentrations (2ng, 10ng, 50ng) of five primers
- The range of concentrations will ensure that mutagenesis is not robust and will yield mutants with a different number and combinations of delta S gene mutations



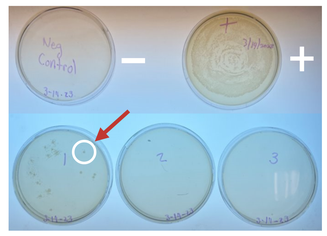
SARS-CoV-2 S Gene Mutagenesis

- 3 Mutagenesis reactions were performed using the Quikchange Multi-Site Directed Mutagenesis Kit
- 5 Primers (L452R, T478K, D614G, P681R, and D950N) at 3 different concentrations (2ng, 10ng, 50ng) were made for each mutagenesis reaction
- Primers were run in thermocycler with DNA template containing alpha S gene (Addgene pGBW-m4137382)
- Mutagenesis reaction products were transformed into competent E. coli cells by heat shock and plated on LB agar with antibiotic selection
- Colonies that contain a mutant S gene were selected and purified using QIAprep Spin Miniprep Kit
- Plasmids samples were sent for sequencing analysis to confirm S gene mutagenesis success



Results and Future Direction

- Harvested 24 colonies of bacteria containing S gene containing plasmids
- Sent plasmid samples in for sequencing; results are currently pending
- If mutations were successful, further studies will include: Immune cell activation assays using THP-1 reporter cell lines (InvivoGen) to investigate NF-κB expression and cell entry kinetics to investigate the rate of SARS-CoV-2 infection



Acknowledgments

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