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Investigating Conserved Sequence in the SARS-CoV-2 Genome as Novel Vaccine Immunogen

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BACKGROUND

- **Goal:** Investigate the highly conserved replication transcription complex (RTC) as a potential broadly protective coronavirus immunogen.

The Omicron variant is the most recent in a long line of SARS-CoV-2 variants of concern (VOC), highlighting the need to investigate novel, broadly conserved coronavirus immunogens for use in a pancoronavirus vaccine. Multiple VOC have evolved increased levels of immune evasion against natural and vaccine-elicited humoral immunity, highlighting the importance of cellular immunity (1). Recently, multiple studies have identified pre-pandemic T-cells targeting the highly conserved RTC as associated with protection or milder SARS-CoV-2 infection (2, 3). This study investigates the vaccine potential of the highly conserved RTC in response to studies identifying RTC targeting T-cells as associated with asymptomatic to mild SARS-CoV-2 infection.

METHODS

- CoV.Con Immunogen generated by aligning coronavirus sequences to determine most conserved region.
- C57BL/6J K18 ACE2 carrier mice and BALB/c mice were immunized intramuscularly with 10^9 RhAd52.CoV.Con and boosted four weeks later.
- RhAd52.CoV.Con was tested in combination with suboptimal dose of RhAd52 expressing SARS-CoV-2 spike with a diproline mutation (RhAd52.S.pp). Suboptimal dose was determined by administering a dilution series of RhAd52.S.pp and measuring spike ELISA binding antibodies (Fig 1) and comparing to previous suboptimal antibody titers (data not shown).

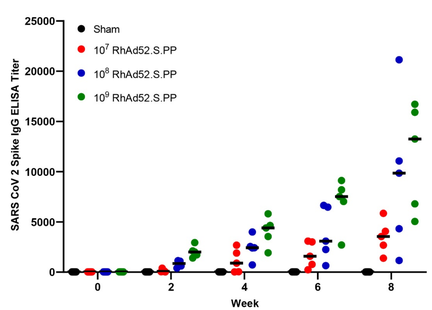


Figure 1: Serum spike ELISA following RhAd52.S.pp vaccination in BALB/c mice.

RESULTS

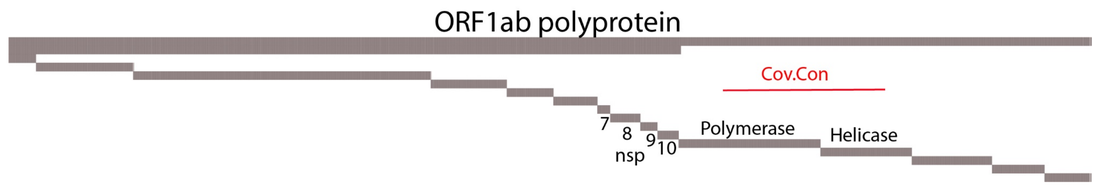


Figure 2: CoV.Con immunogen location in SARS-CoV-2 Genome

- Investigated vaccine immunogen potential of RTC (CoV.Con) (Fig 1).
- Generated Adenovirus serotype 26 (Ad26.CoV.Con) and Rhesus Adenovirus serotype 52 (RhAd52.CoV.Con) viral vectors expressing the CoV.Con immunogen.
- Demonstrated cellular immunogenicity in BALB/c and C57BL/6J K18 ACE2 carrier mice through ELIspot (Fig 2A and B) and intracellular cytokine stain (ICS) (Fig 2C and D).
- BALB/c mice cellular immunity showed bias towards *nsp12,* while C57BL/6J K18 ACE2 carrier mice showed bias towards *nsp13*.
- RhAd52.CoV.Con vaccinated BALB/c mice were challenged with mouse adapted SARS-CoV-2 (4,5) protection against weight loss was minimal.
- RhAd52.CoV.Con showed minimal protection against viral replication in PFU assay.
- Increased RhAd52.S.pp protection was observed from 4 to 8 weeks post immunization corresponded to increased spike antibody binding titers (Fig 1).

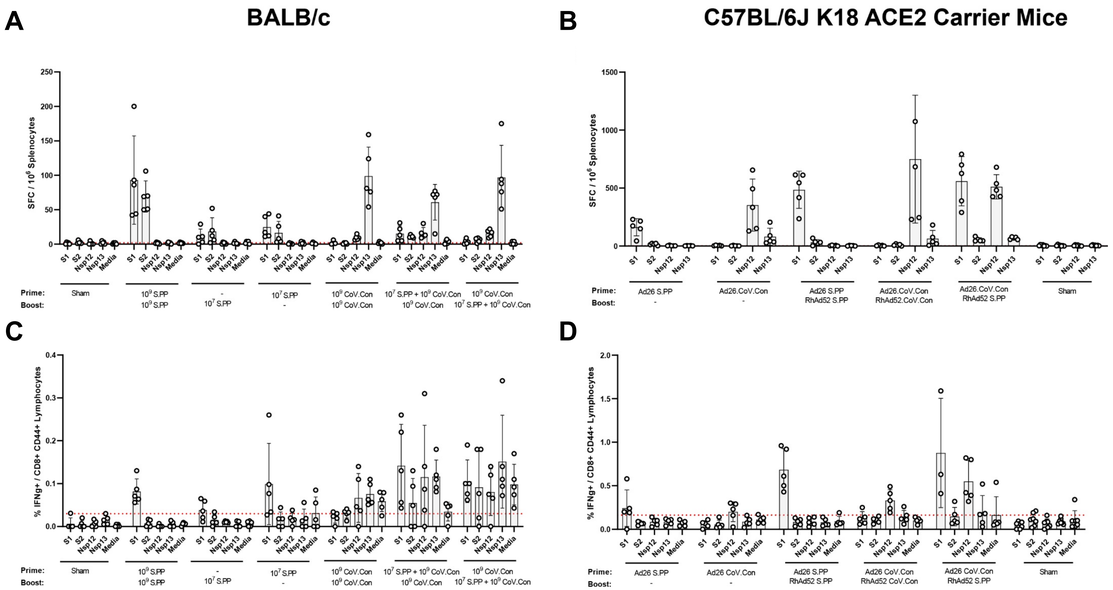


Figure 3: A-B. ELIspot from vaccinated mice. C-D Intracellular cytokine stain on lung immune cells.

CONCLUSIONS

RTC specific T-cells have been associated with protection and/or reduced severity of SARS-CoV-2 infection (2,3). Making it imperative to investigate this region as a potential broadly protective SARS-CoV-2 immunogen. While Ad vectors expressing CoV.Con were immunogenic, in the mouse adapted SARS-CoV-2 model we saw minimal protection against weight loss and virus replication (Plaque Assay). This may be due to limitation in the mouse model. In the mouse adapted SARS-CoV-2 challenge model disease naturally resolves after four days, in comparison human disease extends to about two weeks (22, 23). This could make reduction in disease severity more difficult to observe.

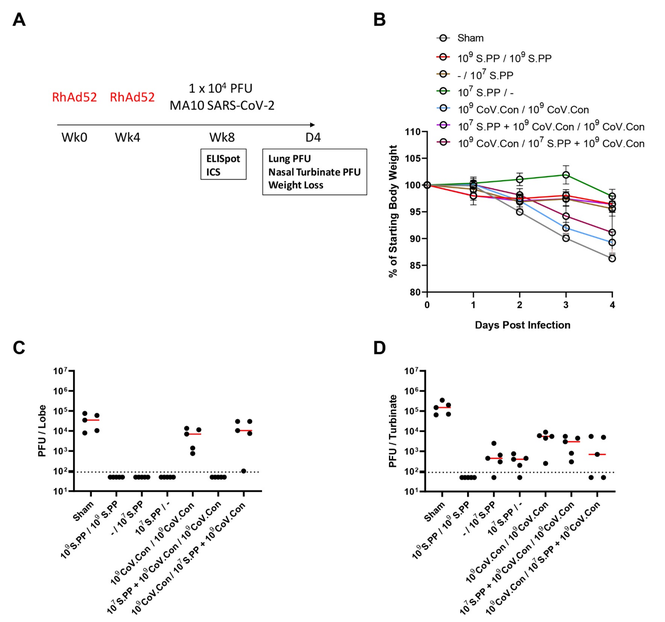


Figure 4: A. Mouse challenge timeline. B. Mouse weight recorded after challenge with mouse adapted SARS-CoV-2. C. Day 4 post challenge PFU results from mouse lower respiratory tract. D. Day 4 post challenge PFU results from mouse upper respiratory tract.

Citations

1. Liu L, *et al.* 2021. Striking Antibody Evasion Manifested by the Omicron Variant of SARS-CoV-2. bioRxiv doi:10.1101/2021.12.14.472719:2021.12.14.472719.
2. Mallajosyula V, *et al.* 2021. CD8⁺ T cells specific for conserved coronavirus epitopes correlate with milder disease in patients with COVID-19. Science Immunology 6:eabg5669.
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