Early Antibody Responses to SARS CoV-2 Infection in Non-Human Primates

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Introduction

- The widespread detrimental effects of the COVID-19 pandemic caused by SARS CoV-2 virus has made investigating the pathogenesis of infection and development of therapeutics a pressing matter.
- As of September 2020, COVID-19 has been linked to the deaths of over 200,000 Americans and infected over 7 million with the respiratory illness.
- Currently, antibody responses following COVID-19 infection are poorly understood. An increased understanding of antibody responses following infection may reveal implications for serological testing in early stages of COVID-19 infection.
- Non-human primate (NHP) models for COVID-19 are needed to characterize virus-specific immune responses under controlled conditions.

This project is the first to assess the earliest primary serum antibody responses that are generated in NHP after infection with the SARS CoV-2 virus.

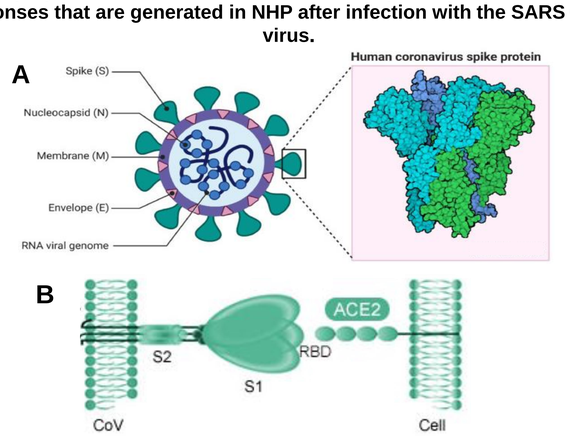

Figure 1: A. Structure of SARS-CoV-2 virion. B. Binding of spike protein to host ACE-2 receptor.

Methods

Experimental Design:

- 6 Indian rhesus macaques were inoculated with 6 x 10^6 infectious units of SARS-CoV-2 by a combination of three routes:
  - Intranasal (2 x 10^6 infectious units)
  - Intratracheal (2 x 10^6 infectious units)
  - Intraocular (2 x 10^6 infectious units)
- Serum samples were collected prior to inoculation and, at intervals, between days 2 and 10 then again at the time of necropsy to measure antibodies at each of these time points.

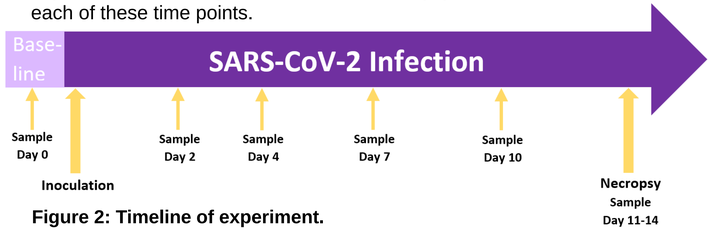

Figure 2: Timeline of experiment.

Bioplex Antibody Assay: SARS-CoV-2 spike subunit 1 (S1), spike subunit 2 (S2), and nucleocapsid (NC) proteins were conjugated to magnetic beads, each embedded with a unique ratio of two infrared dyes (termed "regions"). Beads were incubated with serum samples from monkeys inoculated with virus. Bound SARS-specific antibodies were detected using biotinylated anti-rhesus IgG, IgM, or IgA secondary antibodies and fluorescent avidin-phycoerythrin (PE) in separate assays to measure the concentration of each antibody isotype present in each sample. A calibrated reference was included to generate a standard curve.

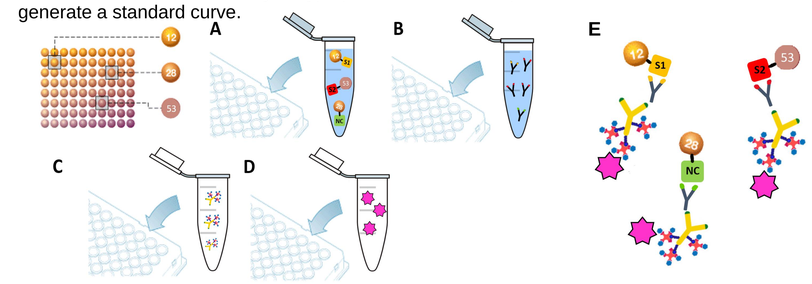

Figure 3: Schematic of the Bioplex assay. **A.** Addition of S1, S2, and NC viral proteins covalently bound to magnetic beads, regions 12, 53, and 28, respectively. **B.** Addition of rhesus serum samples. **C.** Addition of biotinylated secondary antibodies against rhesus IgG, IgM, or IgA. **D.** Addition of fluorophore, avidin-PE. **E.** Median fluorescence intensity associated with each bead region in each well is recorded by the Bioplex machine.

Results

SARS-CoV-2 Specific Antibodies by Isotype

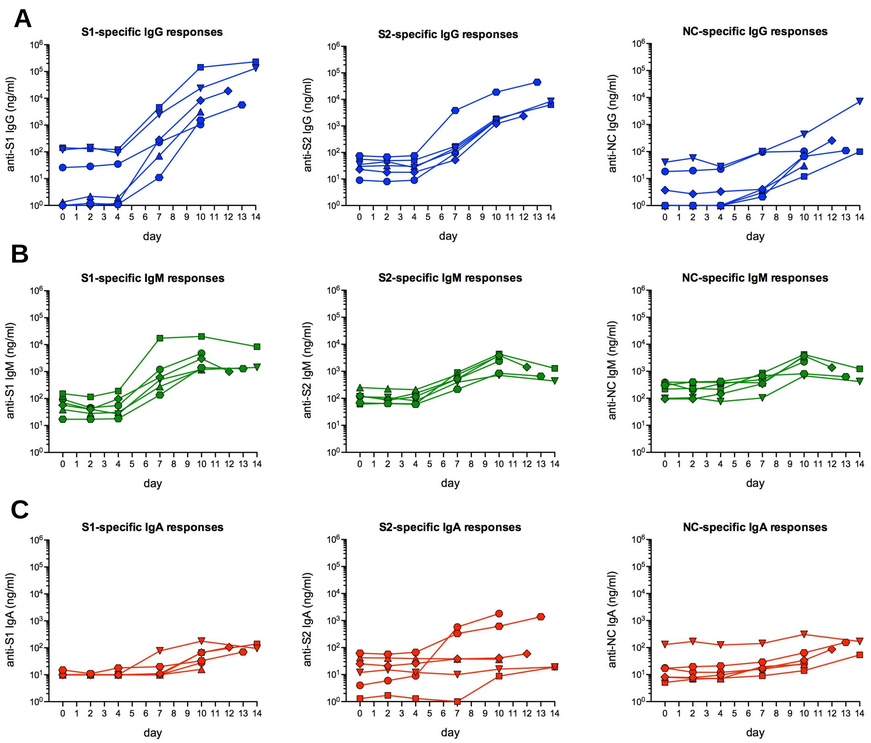

Figure 4: SARS-CoV-2 Specific Antibodies by Isotype. A. Serum IgG responses against S1, S2, and NC proteins throughout course of experiment. **B.** Serum IgM responses against S1, S2, and NC proteins. **C.** Serum IgA responses against S1, S2, and NC proteins.

Fold Increases in SARS-Cov-2 Specific Antibodies

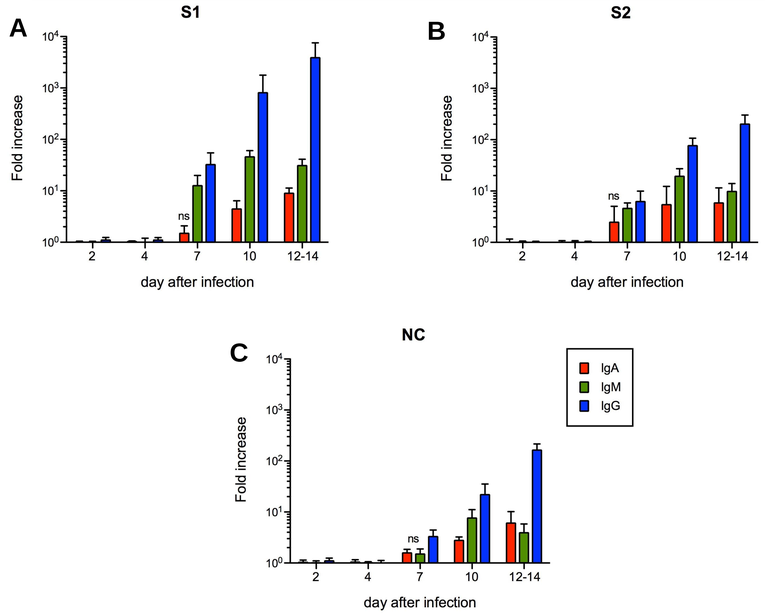

Figure 5: Fold increases in SARS-CoV-2 Specific Antibodies. Fold increases were calculated by dividing the post-infection concentration of antibody in each animal by the pre-infection value. Shown are the mean fold increases in A. anti-S1, B. anti-S2, and C. anti-NC antibodies for each isotype. ns= not significant (<3-fold). All increases on days 7 - 14 were significant.

Conclusions

- As noted in human studies, Spike-specific IgG and IgM antibodies appeared in serum simultaneously.
- Both IgG and IgM antibody responses to Spike protein were readily detected 7 days after infection, but IgG antibodies to NC were very low and significant anti-NC IgM was not found at this time.
- Significant IgA responses were not observed until day 10.
- From day 10 to day 14, SARS-specific IgM declined but IgA increased, suggesting class-switching to IgA was occurring.
- The antibody response to all viral proteins was dominated by IgG.
- **Overall, these results suggest that serological tests based on the presence antibodies to both Spike and NC proteins may not yield positive results until 10 days after infection.**

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