Bovine Immunoglobulin Inhibits the Binding of ACE2 to COVID-19 Spike Protein

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Objective

Determine if serum-derived bovine immunoglobulin (SBI) can bind to the SARS-CoV-2 RBD protein and disrupt RBD-ACE2 binding.

Methods

RBD-SBI Binding ELISA

ELISA was developed to determine SBI binding to RBD. A 96-well plate was coated with RBD, washed, blocked, and incubated with SBI. Detection was performed using HRP-conjugated antibodies against bovine IgG, IgA, or IgM. The reaction was visualized by the addition of TMB and stopped with sulfuric acid (H2SO4). Absorbance was read at 450 nm using a microplate reader. Control experiments were done to ensure specific binding of Ig to RBD protein (Figure 1 B-C).

RBD-ACE2 ELISA

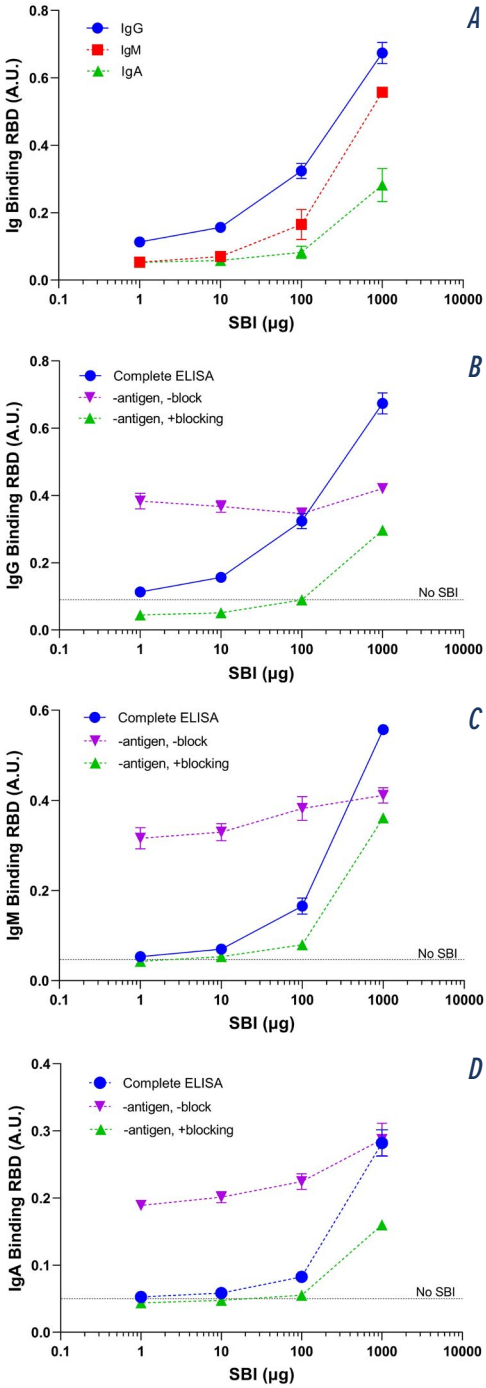
To determine if SBI disrupts ACE2-RBD binding, a second ELISA was developed. Again, a plate was coated with RBD, washed, blocked, and incubated with rhACE2. Detection of the rhACE2 protein was performed using an anti-HisTag-HRP.

Based upon the rhACE2 standard curve (Figure 2 A), the 40 ng/well ACE2 condition was chosen for evaluating SBI treatments on inhibition of ACE2-RBD binding. Prior to incubation with rhACE2, SBI, TBS or fish gelatin (FG) treatments were added to the wells at concentrations of 1-5 mg/well. Fish gelatin was used as negative control protein. Percent inhibition of RBD-ACE2 binding was quantified as follows:

$$\text{Percent inhibition} = 100 \times \left(1 - \frac{\text{OD}_{450\text{nm}}\ \text{Treatment}}{\text{OD}_{450\text{nm}}\ \text{No Treatment}}\right)$$

All components of the ELISA were individually coated in wells and probed with anti-HisTag-HRP (Figure 2 B) to ensure the specificity of the HisProbe-HRP.

Figure 1



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Results

- Absorbance increased proportionally to SBI concentration for IgG, IgM and IgA isotypes, demonstrating binding of immunoglobulins to immobilized RBD protein. (Figure 1 A)
- Control experiments verified effective blocking and specific binding and detection of SBI IgG, IgM and IgA to RBD protein. (Figure 1 B,C)
- SBI treatments reduce binding of ACE2 to RBD proportionally to SBI concentration. Percent inhibition increased from 5.6% to 31.4% for the treatments in Figure 3.
- FG negative control treatments did not inhibit ACE2 binding to RBD.
- Paired t-test determined SBI and FG treatment groups to be statistically different ($p<0.01$).

Conclusion

- Immunoglobulins in SBI bind to SARS-CoV-2 spike protein.
- SBI inhibits the binding of ACE2 to SARS-CoV-2 spike protein.
- Binding and neutralization of RBD by SBI is likely driven by cross reactivity of immunoglobulins against bovine coronavirus and SARS-CoV-2 spike proteins.
- Further in vitro work is necessary to determine if SBI's disruption of RBD-ACE2 binding can reduce SARS-CoV-2 entry into epithelial cells.

Figure 2

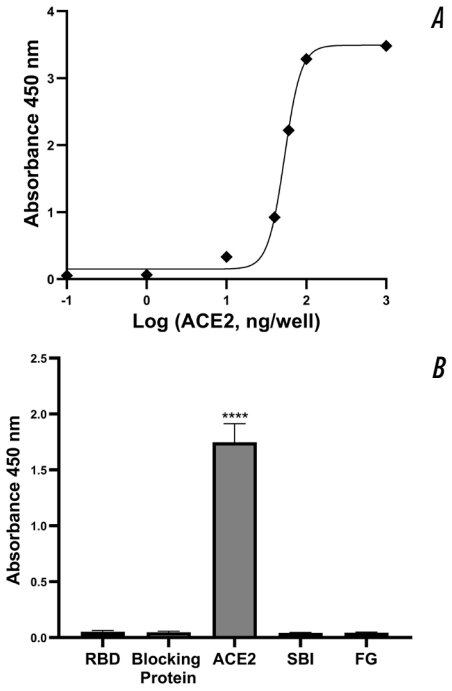
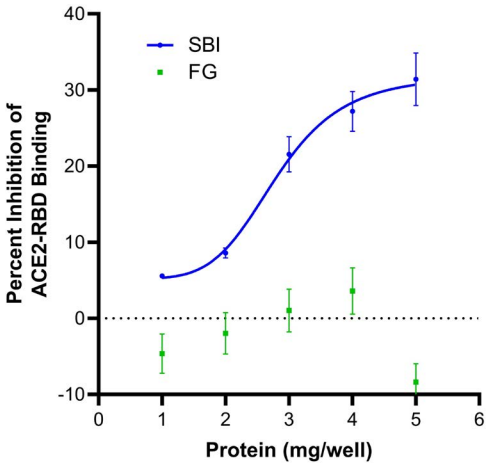


Figure 3



Figures

Figure 1 - Bovine IgG, IgM, and IgA each specifcally binding to RBD protein (Panel A). The binding affinities were qualitatively demonstrated using a modified ELISA technique. ELISA control conditions (B-D) confirmed specific binding of SBI Ig (Complete ELISA). SBI Ig had a higher affinity for RBD protein (Complete ELISA) when compared to the blocking protein (-antigen, +block). Effective blocking is demonstrated by comparing wells treated with blocking protein (-antigen, +block) to wells not treated with blocking protein (-antigen, -block). Specific binding of the detection antibodies is demonstrated by comparison of Complete ELISA curve to No SBI line. Error bars represent $\mp$one standard deviation from triplicate data. Absorbance units (A.U.) measured at 450 nm.

Figure 2 - An RBD-ACE2 binding ELISA was developed to test for the ability of SBI to act as an antagonist. An ACE2 curve was established to verify ELISA performance and to determine assay sensitivity (Panel A). To determine specificity of the detection antibody for rhACE2, the HisProbe-HRP antibody was tested against each ELISA component (Panel B). Error bars represent standard deviation and **** indicates $p<.0001$.

Figure 3 - SBI inhibits binding of ACE2 and SARS-CoV-2 spike protein. The fish gelatin (FG) control showed no inhibition of ACE2-RBD binding. Error bars represent standard deviation from mean absorbance measurements used to calculate percent inhibition.