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Increased Antibody Avidity and Cross-Neutralization of Severe Acute Respiratory Syndrome Coronavirus 2 Variants by Hyperimmunized Transchromosomic Bovine–Derived Human Immunoglobulins for Treatment of Coronavirus Disease 2019

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Passive antibody immunotherapeutics directed against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are promising countermeasures for protection and treatment of coronavirus disease 2019 (COVID-19). SARS-CoV-2 variants of concern (VOCs) and variants of interest (VOIs) can impact the clinical efficacy of immunotherapeutics. A fully human polyclonal antibody immunotherapeutic purified from plasma of transchromosomic (Tc) bovines hyperimmunized with SARS-CoV-2 WA-1 spike (SAB-185) is being assessed for efficacy in a phase 2/3 clinical trial when different circulating SARS-CoV-2 variants predominated. We evaluated antibody binding, avidity maturation, and SARS-CoV-2 VOCs/VOIs virus-neutralizing capacity of convalescent plasma compared with different lots of SAB-185 and individual Tc bovine sera sequentially obtained after each vaccination against Alpha, Epsilon, Iota, Gamma, Beta, Kappa, and Delta variants. In contrast to convalescent plasma, sera and SAB-185 derived from hyperimmunized Tc bovines demonstrated higher antibody avidity and more potent cross-neutralizing activity of VOCs/VOIs. Thus, SAB-185 is a potential promising therapeutic candidate for the treatment of patients infected with SARS-CoV-2 variants.

Keywords. COVID-19; SARS-CoV-2; immunoglobulins; antibody therapy; treatment.

Emergency Use Authorized or phase 2/3 clinical stage passive immunotherapeutic antibody products that have been or are used to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) include polyclonal antibody (pAb) derived from convalescent plasma (CP) from individual donors or immune intravenous immunoglobulin (IVIG) produced from pooled lots of large numbers of immune plasma from convalescent donors [1, 2], and monoclonal-based therapeutics that target 1 or 2 epitopes on the SARS-CoV-2 spike protein. Recently, mutant (or variant) SARS-CoV-2 strains with increased infectivity and virulence that evade endogenous and/or exogenous polyclonal or monoclonal antibodies have emerged [3, 4]. These SARS-CoV-2 variants have been classified by the World Health Organization as variants of concern (VOCs) or variants of interest (VOIs), with the Delta variant becoming dominant nationally and globally (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/) [5, 6].

With the emergence of SARS-CoV-2 variants and continuous waves of pandemic cluster outbreaks globally, therapeutic antibody products with high neutralizing titer and the capacity to neutralize emerging SARS-CoV-2 variants are critically required for effective treatment of coronavirus disease 2019 (COVID-19). It was observed that SARS-CoV-2 vaccination elicited broad cross-neutralization activity against emerging SARS-CoV-2 variants compared with convalescent COVID-19 sera [7, 8]. Therefore, we hypothesized that hyperimmunization could induce high-avidity cross-neutralizing human antibodies that could be harnessed for manufacturing of hyperimmunoglobulins with broader coverage of emerging SARS-CoV-2 variant strains.

A hyperimmune anti–SARS-CoV-2 pAb product (SAB-185) is in late-stage clinical development. This product was produced from a transchromosomic (Tc) bovine platform that can produce anti-target human polyclonal immunoglobulin Gs (IgGs) in large quantities after hyperimmunization with the target antigen(s) [9, 10]. Tc bovines have been generated in which both bovine immunoglobulin heavy chain loci and the lambda

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light chain locus were homozygously inactivated by gene targeting. Previously, human pAb IgGs against Middle East respiratory syndrome coronavirus (MERS-CoV) and *Mycoplasma hominis* were generated in Tc bovines, and both pAb products were shown to be safe and nonimmunogenic in phase 1 or 1b clinical trials [9, 11]. SAB applied this technology to develop a therapeutic treatment for COVID-19, termed SAB-185 [8–10], which has been evaluated in phase 1 and 2 clinical trials and is under evaluation in a phase 3 clinical trial (ClinicalTrials.gov identifiers NCT04468958 and NCT04469179).

To produce SAB-185, the sequence encoding the extracellular domain of the Wuhan- strain spike protein was cloned into a plasmid DNA (pDNA) construct, and recombinant protein was produced using an ExpiSf9 expression system. After hyperimmunization against the spike protein using a prime (pDNA)–boost (protein) strategy, SAB-185 was purified from pooled Tc bovine plasma. While the SARS-CoV-2 VOCs and VOIs were found to have decreased susceptibility to 1 or more types of postinfection human plasma–generated antibody treatments [12–18], the impact of such variants on SAB-185 required investigation.

In this study, we determined the neutralization capacity of the Tc bovine sera and different lots of SAB-185 against the immunization-homologous SARS-CoV-2 WA-1 strain and several VOCs/VOIs (B.1.1.7 [Alpha], B1.351 [Beta], P.1 [Gamma], B.1.429 [Epsilon], B.1.617.2 [Delta], B.1.526 [Iota], and B.1.617.1 [Kappa]) in a pseudovirion neutralization assay (PsVNA). In addition, we analyzed antibody binding titer and antibody avidity maturation of the Tc bovine sera and different lots of SAB-185 against prefusion spike trimers derived from the WA-1, Alpha, and Beta strains by surface plasmon resonance (SPR)–based antibody kinetics assay. As comparator, CP samples from recovered COVID-19 patients were evaluated, as endogenous anti–SARS-CoV-2 antibody avidity to prefusion spike has been reported to provide clinical benefit to human against COVID-19 [19–22].

MATERIALS AND METHODS

Study Design

Tc bovine sera tested in the study were generated by immunization of Tc bovines with 2 doses of plasmid DNA encoding spike protein of SARS-CoV-2 WA-1 strain at a 3-week interval, followed by 5 doses of WA-1 recombinant spike protein at 4-week intervals. Sera from 2 hyperimmunized bovines were collected after each immunization (V1–V7). Three samples of purified human immunoglobulin were produced from plasma collected at postimmunization V3–V5. SAB-185 (V3) and SAB-185 (V4) were purified from pooled V3 plasma and pooled V4 plasma, respectively. SAB-185 (V3–V5) was purified from pooled V3, V4, and V5 plasma. For purification, pooled Tc bovine plasma was fractionated by caprylic acid. The fractionated sample containing Tc bovine–derived human IgG was further purified by affinity chromatography, first using an anti-human IgG kappa light chain–specific column to remove bovine plasma proteins, and second, by passing through an anti-bovine IgG heavy chain–specific affinity column to further remove residual bovine IgG or chimeric IgG (containing bovine heavy chain and human kappa light chain) molecules. The animal care and use protocol meets National Institutes of Health (NIH) guidelines and complied with all ethical regulations.

Seven CP lots were obtained from recovered hospitalized COVID-19 patients between May and July 2020, prior to emergence of Alpha or other VOCs. The CP was collected from unselected plasma donors in the United States (US) at least 30 days after recovery of disease.

Ethics Statement

This study was approved by the US Food and Drug Administration's Research Involving Human Subjects Committee (RIHSC #2020-04-02). Samples were collected from convalescent patients who provided informed consent to participate in the study. All assays performed fell within the permissible usages in the original informed consent.

Lentivirus Pseudovirion Neutralization Assay

Pseudovirion production and PsVNA was described in previous publications from our laboratory against SARS-CoV-2 WA-1 and variants, including B.1.1.7 (Alpha), B.1.429 (Epsilon), B.1.526 (Iota), P.1 (Gamma), B.1.351 (Beta), B.1.617.1 (Kappa), and B.1.617.2 (Delta) [19, 21, 23]. Pseudovirion used in the study were bearing the SARS-CoV-2 spike from the WA-1 strain and with spike mutations shown in Supplementary Table 1. The PsVNA using 293-ACE2-TMPRSS2 cell line was described previously [4, 19, 23, 24]. All sera were heat-inactivated prior to use in the assay.

The limit of detection for the neutralization assay is 1:10. Neutralizing antibody titers ($PsVNA_{50}$) were measured as sample dilution that resulted in 50% reduction in viral titers. A $PsVNA_{50}$ titer >1:60 was used as a seropositive cutoff based on current understanding of neutralizing antibody as correlate of protection against COVID-19 [25].

Proteins

The SARS-CoV-2 spike plasmid encodes residues 1–1208 of the genetically stabilized prefusion 2019-nCoV_S-2P spike ectodomain fused to 8xHisTag, which was a kind gift from Barney Graham (earlier at Vaccine Research Center, NIH) [26]. This expression vector was used to transiently transfect FreeStyle293F cells (ThermoFisher; catalog number R79007) using polyethylenimine (Polysciences Inc). Protein was purified from filtered cell supernatants using StrepTactin resin (Cytiva, catalog number 29401326) and subjected to additional purification by size-exclusion chromatography in phosphate-buffered saline (PBS). HEK-293 mammalian cells producing SARS-CoV-2

prefusion spike of B.1.1.7 and B.1.351 were purchased from Acros Biologicals Inc. The native receptor-binding activity of the spike proteins was determined by binding to the human ACE2 protein.

Antibody-Binding Kinetics to SARS-CoV-2 Spike by SPR

SARS-CoV-2 antibody-binding kinetics by SPR was described in previous publications from our laboratory [19, 21, 23, 27]. In brief, serially diluted 5, 1, or 0.2 mg/mL of Tc bovine sera or purified SAB-185 or a 10-, 50-, or 250-fold dilution of CP in BSA-PBST buffer (PBS pH 7.4 buffer with Tween-20 and bovine serum albumin [BSA]) was injected at a flow rate of 50 µL/minute (120 seconds' contact duration) for association, and disassociation was performed over a 600-second interval using a ProteOn SPR (Bio-Rad). Responses from the SARS-CoV-2 protein surface were corrected for the response from a mock surface and for responses from a buffer-only injection. The protein density on the chip was optimized to measure monovalent interactions independent of the antibody isotype. Total antibody binding was calculated with BioRad ProteOn manager software (version 3.1). All SPR experiments were performed twice, and the researchers performing the assay were blinded to sample identity.

Antibody off-rate constants, which describe the stability of the antigen-antibody complex (ie, the fraction of complexes that decays per second in the dissociation phase), were determined directly from the human polyclonal sample interaction with recombinant purified SARS-CoV-2 proteins using SPR, only for the sensorgrams with Max resonance units (RU) in the range of 5–150 RU and calculated using the BioRad ProteOn manager software for the heterogeneous sample model as previously described [19, 21, 23, 27–29]. Off-rate constants were determined from 2 independent SPR runs. Previously we confirmed that antigen-antibody binding off-rate of purified IgG from plasma and fragment antigen-binding (Fab) fragments with prefusion spike under optimized conditions in SPR were very similar when adjusted for molecular weight of the bound IgG and Fab molecules. The variation for each sample in duplicate SPR runs was <5%. The data shown are the average value of 2 experimental runs.

RESULTS

Neutralization Capacity of Tc Bovine Sera and Convalescent Plasma Against SARS-CoV-2 WA-1 and VOCs/VOIs

Two Tc bovine animals (2378 and 2395) were each immunized with 2 doses (V1 and V2) of plasmid DNA (pDNA) encoding SARS-CoV-2 WA-1 strain spike protein at 3-week intervals, followed by 5 doses (V3–V7) of recombinant spike protein at 4-week intervals to generate fully human, polyclonal immunoglobulin against SARS-CoV-2. Serum and/or plasma samples were collected at 8, 11, or 14 days after each vaccination for antibody analysis. $PsVNA_{50}$ neutralization titers (serum dilution to achieve 50% SARS-CoV-2 reduction in viral titers) for Tc bovine sera were established.

The potencies of Tc bovine sera were determined by PsVNA against the SARS-CoV-2 strains Alpha (B.1.1.7), Epsilon (B.1.429), Iota (B.1.526), Gamma (P.1), Beta (B.1.351), Kappa (B.1.617.1), and Delta (B.1.617.2). The amino acid mutations in the spike protein of VOCs/VOIs used for production of the pseudovirions are shown in Supplementary Table 1. A $PsVNA_{50}$ titer >1:60 was used as a seropositive cutoff based on the correlation between neutralizing antibody and protection against COVID-19 [25].

The neutralization activities of V1–V7 longitudinal Tc bovine heat-inactivated serum samples from 2 Tc bovine were measured by PsVNA against the vaccine-homologous SARS-CoV-2 strain (WA-1) and emerging VOCs/VOIs. Neutralizing antibodies were not detected in the sera after the first 2 pDNA immunizations, V1 and V2 (Figure 1A). Boosting the animals with purified spike protein for the third immunization (V3) generated high titers (mean, 1:4165) against vaccine-homologous WA-1. The $PsVNA_{50}$ fold reduction between the WA-1 strain and the VOCs/VOIs was 2.7- to 13-fold for the different variants. After the fourth immunization, the $PsVNA_{50}$ titers of all Tc bovine sera increased substantially, with $PsVNA_{50}$ >1:1000 against WA-1 and VOC/VOI strains (Figure 1A and Table 1). For V4, a 2.5-fold reduction of $PsVNA_{50}$ titers was observed against Alpha or Epsilon, and 2- to 6-fold reduction against Iota, Gamma, Beta, Kappa, and Delta SARS-CoV-2 strains (Table 1) compared with WA-1. Tc bovine sera collected at V5–V7 exhibited higher $PsVNA_{50}$ titers against WA-1 (range, 1:10 236–1:34 089) compared with SARS-CoV-2 VOCs/VOIs ($PsVNA_{50}$ range, 1:2269–1:32 956) (Table 1). Overall, the $PsVNA_{50}$ titers in Tc bovine sera increased rapidly after V3, peaked at V5, and maintained high levels from V5 to V7.

For comparison, neutralization assays were performed on 7 human CP samples obtained at least 30 days postdischarge from recovered hospitalized COVID-19 patients between May and July 2020, prior to the emergence of VOCs against the panel of pseudovirion SARS-CoV-2 strains (WA-1 and all SARS-CoV-2 variants). Variable $PsVNA_{50}$ titers (1:120–1:1872) were observed for the 7 CP samples against the WA-1 strain with weak to moderate neutralization (Figure 1A and Table 1). CP samples against SARS-CoV-2 VOCs/VOIs demonstrated moderate reduction of $PsVNA_{50}$ titers against Alpha, Epsilon, and Delta variants. However, the majority of the CP samples (5/7) lost their neutralization activity against Gamma, Iota, Kappa, and Beta variants. Additionally, all 7 CP samples were seronegative against the Beta variant. Samples CP-2 and CP-5 did not neutralize any of the VOCs/VOIs, and sample CP-6 only neutralized the Delta variant (Figure 1A and Table 1). Tc bovine sera collected at V3 surpassed the neutralization activity of CP against WA-1 and SARS-CoV-2 variants (Table 1). Compared with Tc bovine serum, the CP lots exhibited greater loss (up to 100-fold) of neutralizing activities against the SARS-CoV-2 variants (Figure 1A and Table 1).

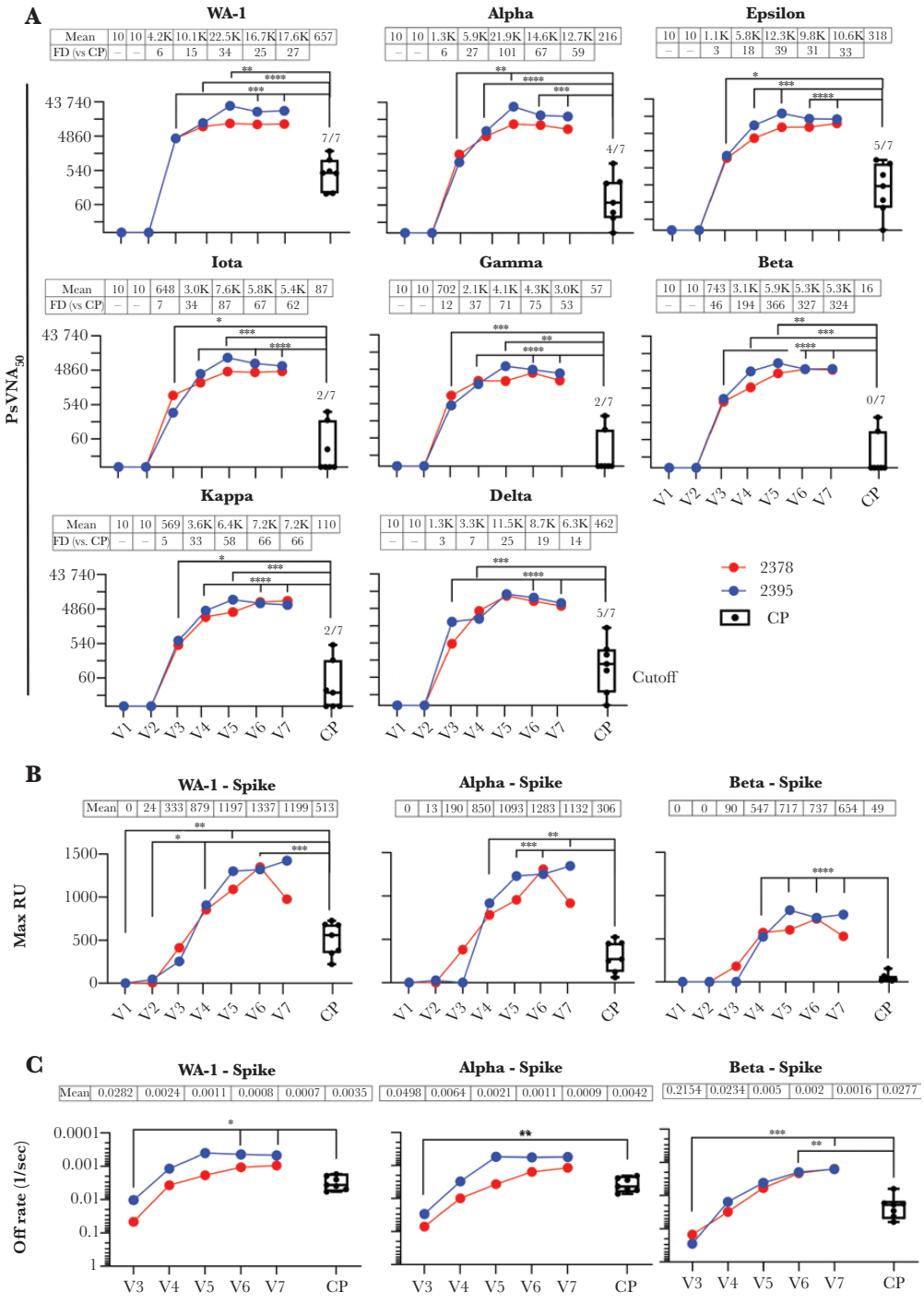

Figure 1. Antibody responses of transchromosomic (Tc) bovine sera compared with convalescent plasma against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) WA-1 strain and variant strains. *A*, SARS-CoV-2 neutralizing antibody titers in Tc bovine sera after each immunization and 7 convalescent plasma (CP) lots are determined by pseudovirion neutralization assay (PsVNA) in 293-ACE2-TMPRSS2 cells with SARS-CoV-2 WA-1 strain and Alpha (B.1.1.7), Epsilon (B.1.429), Iota (B.1.526), Gamma (P.1), Beta (B.1.351), Kappa (B.1.617.1), and Delta (B.1.617.2) variants. Tc bovine sera used in the assay were serially diluted from starting concentration of 1 mg/mL, while convalescent plasma started at original plasma concentration. $PsVNA_{50}$ (50% neutralization titer) for each of the control CP (n =7) and Tc bovine sera (n = 14) were calculated with GraphPad Prism version 9. $PsVNA_{50}$ values lower than the first dilution 1:20 were set as 10 for analysis and graphing purpose. Seropositive cutoff (cutoff) was set at 1:60 $PsVNA_{50}$ titer. Antibody responses from 3 Tc bovines are color coded. $PsVNA_{50}$ of CP lots are shown in black. Seropositive CP lots (out of total 7 CP lots) are presented as box and whisker plots, where boxes extend from 25th to 75th percentile, whiskers show minimum to maximum value, and central band represents the median value for the group. The numbers above the group show the mean value for each group. Fold difference (FD) of $PsVNA_{50}$ titers for Tc bovine sera vs mean CP titers is also shown above in each panel. *B*, Total antibody binding (Max resonance units [RU]) of 1 mg/mL Tc bovine sera (V1–V7) and 1:10 dilution of CP lots to purified prefusion spike of WA-1 spike and Alpha and Beta variants of concern (VOCs) by surface plasmon resonance (SPR). *C*, Antibody avidity (antibody-antigen dissociation rate) of each of Tc bovine serum sample to purified prefusion spike of WA-1 spike and Alpha and Beta VOCs by SPR only for the Tc bovine samples with Max RU in the range of >5 RU as described in the Methods. Numbers above the group show the mean value for each group. Statistical comparisons between Tc bovine titers vs CP titers were performed using R software. Differences were considered statistically significant with a 95% confidence interval when the *P* value was < .05. **P* ≤ .05, ***P* ≤ .01, ****P* ≤ .001, *****P* ≤ .0001.

Table 1. Pseudovirus neutralization assay 50% inhibition ($PsVNA_{50}$) Endpoint Titers of SAB-185 Transchromosomic Bovine Serum and Convalescent Plasma to WA-1 and Variants and Fold Decrease of $PsVNA_{50}$ Against Variants Compared With WA-1 Strain

	$PsVNA_{50}$								Fold Decrease						
SAB (Inhibition Dilution)	WA-1	B.1.1.7 (Alpha)	B.1.429 (Epsilon)	P.1 (Gamma)	B.1.351 (Beta)	B.1.617.1 (Kappa)	B.1.617.2 (Delta)	B.1.526 (Iota)	B.1.1.7 (Alpha)	B.1.429 (Epsilon)	P.1 (Gamma)	B.1.351 (Beta)	B.1.617.1 (Kappa)	B.1.617.2 (Delta)	B.1.526 (Iota)
TcB serum															
2378 V1D0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2378 V2D11	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2378 V3D8	4152.2	1553.7	1013.4	919.9	676.0	489.4	515.5	975.2	2.7	4.1	4.5	6.1	8.5	8.1	4.3
2378 V4D7	8959.4	4902.6	3568.3	2314.9	1674.2	2885.3	4110.7	2188.6	1.8	2.5	3.9	5.4	3.1	2.2	4.1
2378 V5D7	10 888.2	10 776.3	7157.6	2269.3	4060.8	3972.3	10 819.2	4436.8	1.0	1.5	4.8	2.7	2.7	1.0	2.5
2378 V6D7	10 236.6	10 135.1	7316.5	3840.7	5250.5	7512.3	7780.0	4205.2	1.0	1.4	2.7	1.9	1.4	1.3	2.4
2378 V7D7	10 583.2	7737.0	9166.5	2357.3	5107.5	8148.0	5703.8	4494.0	1.4	1.2	4.5	2.1	1.3	1.9	2.4
2395 V1D0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2395 V2D11	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2395 V3D7	4178.7	938.7	1179.7	483.8	809.0	648.5	2079.3	321.4	4.5	3.5	8.6	5.2	6.4	2.0	13.0
2395 V4D7	11 241.2	6851.4	8101.6	1898.0	4615.9	4359.5	2510.8	3826.7	1.6	1.4	5.9	2.4	2.6	4.5	2.9
2395 V5D7	34 089.9	32 955.8	17 408.1	5908.3	7833.2	8756.3	12 103.1	10 729.3	1.0	2.0	5.8	4.4	3.9	2.8	3.2
2395 V6D7	23 149.2	19 007.4	12 327.7	4713.2	5373.3	6931.2	9718.8	7434.5	1.2	1.9	4.9	4.3	3.3	2.4	3.1
2395 V7D7	24 698.7	17 638.7	12 072.9	3739.6	5402.7	6289.1	6873.5	6373.7	1.4	2.0	6.6	4.6	3.9	3.6	3.9
Convalescent plasma endpoint dilution[a]															
CP-1	458.4	240.4	339.9	102.1	42.2	187.6	1002.8	341.8	1.9	1.3	4.5	10.9	2.4	0.5	1.3
CP-2	516.2	69.1	68.2	10.0	10.0	28.1	10.0	10.0	7.5	7.6	51.6	51.6	18.4	51.6	51.6
CP-3	447.6	37.3	166.3	10.0	10.0	10.0	212.5	10.0	12.0	2.7	44.8	44.8	44.8	2.1	44.8
CP-4	1872.2	863.6	705.1	248.8	21.4	497.4	369.7	197.6	2.2	2.7	7.5	87.7	3.8	5.1	9.5
CP-5	119.5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
CP-6	124.0	26.1	42.2	10.0	10.0	10.0	110.9	10.0	4.8	2.9	12.4	12.4	12.4	1.1	12.4
CP-7	1061.5	264.8	895.2	10.0	10.0	23.8	1521.5	31.1	4.0	1.2	106.2	106.2	44.7	0.7	34.1

Abbreviations: CP, convalescent plasma; $PsVNA_{50}$, pseudovirus neutralization assay's 50% inhibitory dilution; TcB, transchromosomic bovine.

[a] $PsVNA_{50}$ values lower than first dilution (1:20) are set to 10. Seropositive cutoff for $PsVNA_{50}$ is 1:60.

Antibody Binding and Avidity Maturation of Tc Bovine Sera to Prefusion Spike of SARS-CoV-2 WA-1, Alpha, and Beta Strains

SARS-CoV-2 neutralizing antibodies target several sites within the spike protein including N-terminal domain, receptor-binding domain, and S2 domain [30]. Key mutations in SARS-CoV-2 VOC/VOI spike protein may lead to decreased neutralization by antibodies following infection or escape from vaccination-generated antibodies [12, 31, 32]. These mutations have been shown to promote neutralization escapes of postinfection, postvaccination sera, monoclonal antibodies, and CP from COVID-19 patients [33, 34]. To assess the impact of key spike protein mutations on vaccination-induced antibodies in Tc bovine sera and postinfection antibodies in the CPs, we evaluated antibody binding to stabilized prefusion spike ectodomain utilizing SPR (Figure 1B). For this analysis, we chose the Alpha VOC prefusion spike as it demonstrates similar neutralization results as with the WA-1 strain. Also, the Beta variant prefusion spike protein was selected because it is the most resistant to postvaccination/infection antibodies [32]. Consistent with $PsVNA_{50}$ results, SPR analysis demonstrated low antibody binding (<24 RU) for the V2 Tc bovine sera samples to the WA-1, Alpha, and Beta prefusion spike proteins (Figure 1B). Longitudinally, antigen-antibody complex formation rapidly increased from V3 onward for all Tc bovine sera against the spike protein of WA-1 and variants reaching peak antibody binding at V6 (Figure 1B). The total antibody binding of V5–V7 Tc bovine sera was significantly higher than the antibody binding of the 7 CP lots against WA-1 spike as well as both spike variants. For Tc bovine sera, antibody binding to the Alpha spike protein had minimal to no loss in activity compared with WA-1 spike; however, binding of CP antibodies decreased 1.7-fold. Consistently, binding antibodies to the Beta prefusion spike reduced approximately 2-fold for Tc bovine sera and >10-fold for the 7 CP samples (Figure 1B).

An SPR-based real-time kinetic assay can be used to determine the dissociation kinetics of antibody bound to the SARS-CoV-2 prefusion spike as a surrogate of antibody avidity. Slower antibody-antigen dissociation rates (off-rates) represent higher avidity antibodies. Antibody affinity maturation is a key aspect of an effective immune response following vaccination or infection, as higher antibody avidity contributes to virus neutralization ability and protection from COVID-19 [19, 21]. In addition to measuring binding antibodies against the prefusion spike of WA-1, Alpha, and Beta, we measured the antibody avidity maturation of longitudinal Tc bovine plasma to these prefusion spike proteins. Previously, we showed that the antibody kinetics measured under optimized SPR conditions of monovalent interactions between antibody-antigen complex were in the SPR binding range of 5–150 RU. This range was similar to the antigen-antibody binding off-rates of the IgG and Fab interaction with protein antigens [19, 21, 23, 29]. The avidity of Tc bovine sera to WA-1 spike increased with each immunization and reached plateau at approximately V5, indicating the impact of repeated Tc bovine immunization on antibody avidity maturation (Figure 1C). The Tc bovine sera binding avidity to the Alpha spike was comparable with the spike of WA-1 and followed the same trend. However, the antibody avidity of the V6–V7 Tc bovine sera to the Beta prefusion spike was 4- to 7-fold lower than with the corresponding WA-1 spike protein, but significantly higher than antibody avidity of CP against the Beta spike (Figure 1C).

Neutralization and Antibody Avidity of Purified SAB-185 current Good Manufacturing Practice Lots Against SARS-CoV-2 WA-1 and Its Variants

The Tc bovine plasma were used to generate fully human, polyclonal immunoglobulin Tc bovine-human IgG (hIgG) current Good Manufacturing Practice (cGMP) lots against SARS-CoV-2, termed SAB-185. We selected multiple purified samples representative of SAB-185 for further analysis: SAB-185 (V3) obtained from V3 plasma (after 3 immunizations), SAB-185 (V4) from V4 plasma, and SAB-185 (V3–V5) from pooled V3–V5 plasma.

We first evaluated the neutralization capability of SAB-185 purified lots using PsVNA. SAB-185 (V3) showed lower $PsVNA_{50}$ (endpoint at 1.41 μg/mL) to the WA-1 strain compared with the SAB-185 (V4, endpoint at 0.14 μg/mL) and SAB-185 (V3–V5, endpoint at 0.03 μg/mL) lots, which demonstrated high potency (Figure 2A and Supplementary Table 2). SAB-185 (V3) had $PsVNA_{50}$ ranging between 3 and 7 μg/mL against Alpha, Epsilon, and Iota variants but relatively lower $PsVNA_{50}$ against Gamma, Beta, Kappa, and Delta VOCs (13.4 μg/mL, 52 μg/mL, 21 μg/mL, and 13.1 μg/mL, respectively). Notably, the 2 hyperimmunized SAB-185 samples manufactured from later plasma postvaccination, SAB-185 (V4) and SAB-185 (V3–V5), purified from the high neutralization titer/high antibody avidity plasma, retained $PsVNA_{50}$ of <1.25 μg/mL against all 7 SARS-CoV-2 VOCs/VOIs (Figure 2A and Supplementary Table 2). These data are in agreement with the observed increase in virus neutralization titers observed in the individual Tc bovine animals in late postimmunization bleeds (V4–V7; Figure 1A). This correlation suggested that the high neutralization titer/high antibody avidity products have better potency to neutralize and are likely to provide cross-protection against SARS-CoV-2 VOCs/VOIs.

Antibody Binding and Avidity of Purified SAB-185 cGMP Lots to Prefusion Spike Derived From SARS-CoV-2 WA-1, Alpha, and Beta Strains

To assess the antibody quality in the purified SAB-185 cGMP lots, the total antibody binding and avidity to prefusion spike of WA-1, Alpha, and Beta variants were examined with SPR analysis. The hyperimmunized SAB-185 (V4) and SAB-185 (V3–V5), which had high neutralization titers, exhibited strong antibody binding and high avidity to all prefusion spike proteins (Figure 2B and 2C). SAB-185 (V3) showed approximately

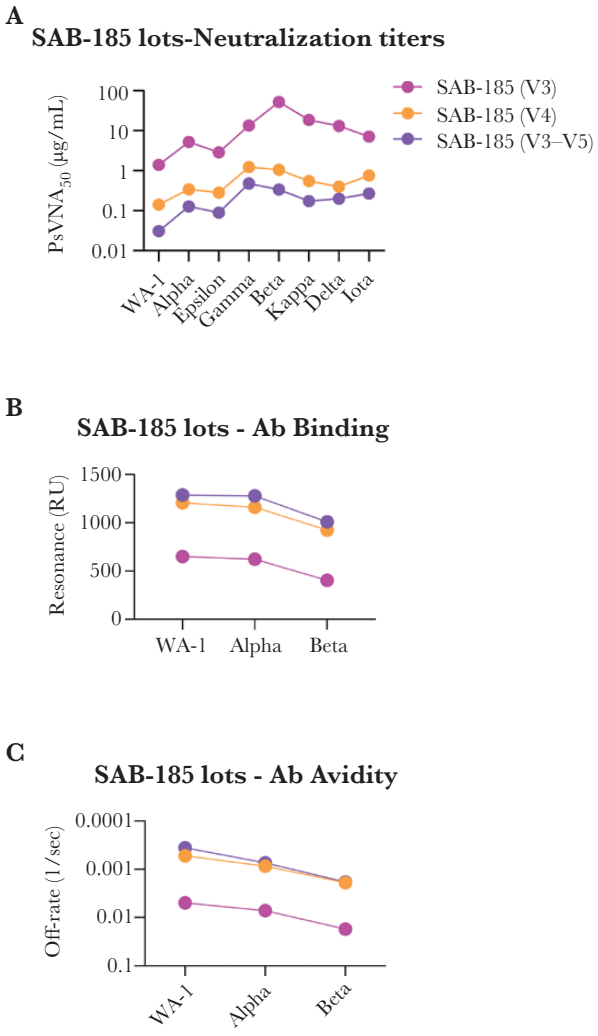

Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) prefusion spike binding antibodies and neutralizing antibody (Ab) responses of purified SAB-185 current Good Manufacturing Practice (cGMP) lots against SARS-CoV-2 WA-1 strain and variant strains. *A*, SARS-CoV-2 neutralizing antibody titers in 3 samples of purified SAB-185 produced from transchromosomic (Tc) bovine plasma are determined by pseudovirion neutralization assay (PsVNA) using 293-ACE2-TMPRSS2 cells with SARS-CoV-2 WA-1 strain and Alpha (B.1.1.7), Epsilon (B.1.429), Iota (B.1.526), Gamma (P.1), Beta (B.1.351), Kappa (B.1.617.1), and Delta (B.1.617.2) variants. SAB-185 (V3) was purified from V3 plasma, while cGMP lots SAB-185 (V4) and SAB-185 (V3–V5) were from V4 and pooled V3–V5 plasma, respectively. The purified SAB-185 samples were serially diluted from starting concentration of 1 mg/mL. $PsVNA_{50}$ (50% neutralization titer) was calculated with GraphPad Prism version 9. Ab responses from 3 purified SAB-185 samples were color coded for all figures. *B*, Total Ab binding (Max resonance units [RU]) of 1 mg/mL of SAB-185 samples to purified prefusion spike of WA-1 spike and B.1.1.7 and B.1.351 variants of concern (VOCs). *C*, Ab avidity (Ab-antigen dissociation rate per second) of each of SAB-185 sample to purified prefusion spike of WA-1 spike and Alpha and Beta VOCs by surface plasmon resonance as described in the Methods.

2.2-fold lower antibody binding (Figure 2B) and 9.6-fold lower antibody avidity (0.00496 per second) against WA-1 prefusion spike than did the other 2 hyperimmunized SAB-185 IgG (0.00053 and 0.00036 per second, respectively) (Figure 2C). All 3 SAB-185 IgG samples showed minimum to no decrease of antibody binding to the Alpha spike (Figure 2B) and <2-fold decrease in antibody avidity (increased dissociation rate) to the Alpha spike compared with the WA-1 spike protein (Figure 2C). Antibody binding of all 3 SAB-185 lots to the Beta prefusion spike decreased by approximately 1.8-fold compared with the WA-1 spike. In addition, the antibody avidity to the Beta prefusion spike decreased by 3- to 4-fold compared with the WA-1 spike.

DISCUSSION

With the continued spread of SARS-CoV-2 variants worldwide, an effective therapeutic treatment against SARS-CoV-2 VOCs/VOIs is urgently needed. It is critical to develop and evaluate broadly neutralizing antibody therapeutics against both circulating SARS-CoV-2 strains and the VOCs/VOIs. Tc bovines have been used to produce high-titer and/or high-avidity polyclonal human antibodies against viral targets such as MERS-CoV and Ebola [10, 35–37]. Historically, multiple polyclonal immunoglobulin products have been produced in animals and humans. Animal-derived immunoglobulins have the advantage of being a high-volume source, but the disadvantage of known ability to cause severe hypersensitivity reactions as their heterologous antibodies are highly immunogenic in humans. Human-derived immunoglobulins are generally nonimmunogenic in human recipients but have the limitation of needing to acquire a large number of donors (and plasma) with a sufficient titer against the target of interest. The use of Tc bovines to produce specific human immunoglobulins has the potential of combining the benefits of animals and humans. Prime-boost vaccination strategies with alternate vaccine platforms or different variant antigens and/or adjuvant combinations could be used in this Tc bovine model to potentially generate higher titers of broadly neutralizing human antibodies.

In this study, we assessed the longitudinal antibody responses in sera from Tc bovine animals that were hyperimmunized with the WA-1 spike. The Tc bovine serum was compared with CP samples from recovered COVID-19 patients utilizing assays that measure antibody binding and neutralizing capacity against the vaccine-homologous WA-1 strain and the SARS-CoV-2 VOCs/VOIs. The reduction in Tc bovine serum neutralization against VOCs/VOIs are in line with data from vaccinated human sera following repeated immunizations with SARS-CoV-2 mRNA vaccines (mRNA-1273 [Moderna] or BNT162b2 [Pfizer]) that demonstrated a similar decrease in fold reduction against variants [32]. The antibody-binding, neutralization titers, and antibody avidity all increased against both the SARS-CoV-2 WA-1 strain and the variant strains with each sequential WA-1 spike protein immunization. Sera derived from Tc bovine hyperimmunization exhibits advantages over postinfection antibody products, such as human CP or IVIG products, which often show low antibody avidity and

significant reduction in neutralization to SARS-CoV-2 variants [4, 14, 33].

The SAB-185 purified from hyperimmunized Tc bovine plasma (SAB-185 [V4] and SAB-185 [V3–V5]) demonstrated high antibody binding avidity to SARS-CoV-2 spike of the vaccine-homologous WA-1 strain as well as the stabilized prefusion spike of the Alpha and Beta VOCs. SARS-CoV-2 neutralizing activity measured by PsVNA correlates well with plaque reduction neutralization test with authentic SARS-CoV-2 virus in studies from multiple laboratories [19, 21, 23, 24]. Consistently, the Tc bovine sera and the purified SAB-185 demonstrated high potency for effectively neutralizing both the SARS-CoV-2 WA-1 strain and circulating VOCs/VOIs. SAB-185 is currently being evaluated in clinical studies (ClinicalTrials.gov identifiers NCT04468958 and NCT04469179).

In summary, compared with postinfection human CP lots, both hyperimmunized Tc bovine sera and purified SAB-185 demonstrated high antibody avidity and high neutralizing capacity against emerging VOCs/VOIs: Alpha, Epsilon, Iota, Gamma, Beta, Kappa, and Delta strains. Thus, anti–SARS-CoV-2 purified SAB-185 may likely lead to effective virus neutralization and protection against emerging SARS-CoV-2 strains and could potentially serve as an effective therapy for treatment of patients with COVID-19, even those infected with circulating SARS-CoV-2 VOCs/VOIs.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. Designed research: S. K. Performed research: J. T., G. G., Y. L., and S. K. Samples from transchromosomic bovine and purified SAB-185 products: H. W., T. C. L., K. A. E., C. L. B., and E. J. S. Contributed to writing: J. T. and S. K. All authors contributed to writing, editing, and review of the manuscript.

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Potential conflicts of interest. H. W., T. C. L., K. A. E., C. L. B., and E. J. S. were employees of SAB Biotherapeutics, Inc, at the time of the study and have financial interests in their company. The authors have developed a lead candidate therapeutic antibody (SAB-185) from this work, which would be considered a product of the company intended for further development as a commercial venture. This does not alter the authors' adherence to policies on sharing data and materials. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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