

Equine hyperimmune globulin raised against the SARS-CoV-2 spike glycoprotein has extremely high neutralizing titers

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Abstract

COVID-19 pandemic caused approximately 750,000 deaths and over 20 million confirmed cases of infection by SARS-CoV-2 within 8 months since the emergence of the virus. While there are no vaccines approved and considering the difficulty in meeting the large vaccination demand worldwide, the potential use of passive immunization should be considered based on existing successful therapies against many diseases. Here we demonstrate that hyperimmune globulin preparations raised in horses against the recombinant trimeric spike (S) glycoprotein of SARS-CoV-2 in the prefusion conformation provide very high ELISA titers as well as highly potent neutralizing activity against SARS-CoV-2. Five horses were subcutaneously inoculated for 6 weeks with the recombinant S protein (ectodomain, residues 1-1208). Four out of the 5 horses presented a strong immune response. Considering the average of all 5 horses, ELISA titers above 1:1,000,000 and neutralizing titers (PRNT₉₀) reaching 1:14,604 were observed. When compared with the plasma of three convalescent COVID-19 patients, sera of immunized horses displayed approximately 140-fold higher neutralizing titers measured as PRNT₉₀. To prevent eventual side effects caused by horse antiserum, IgG was digested with pepsin and purified by fractional salt precipitation to eliminate Fc fragments, a process that is industrially used for the production of passive immunization F(ab')₂ concentrates against rabies, tetanus and snake venoms. The high neutralizing titers against SARS-CoV-2 obtained for the unprocessed sera were confirmed for the F(ab')₂ fragments and were 150-fold higher than the PRNT₉₀ neutralizing titers of plasma of three COVID-19 convalescent patients. The great advantage of using the recombinant trimeric S glycoprotein is that it is safe and provides

quick adaptive immunity in horses. Our data show the perspective of using hyperimmune anti-SARS-CoV-2 F(ab')₂ preparations as a passive immunization therapy in humans, similar to therapies that have been safely used for decades against rabies, tetanus and snake venoms.

Key-words: SARS-CoV-2, COVID-19, trimeric spike protein, equine antibodies, F(ab')₂ fragments, hyperimmune globulins

Introduction

The pandemic caused by the SARS-CoV-2 coronavirus, etiological agent of COVID-19, is an urgent health problem in the world, especially in the Americas (<https://covid19.who.int/>). Considering the absence of approved antiviral treatments and vaccines and the uncertainties regarding antibody responses in individuals infected by SARS-CoV-2¹⁻⁴, the COVID-19 scenario seems to be still far from a solution. The SARS-CoV-2 pandemic is imposing devastating consequences for human health and for the global economy. Passive immunization using plasma from COVID-19 convalescent patients has been used as an alternative^{5,6}. However, the heterogeneous response in terms of patient titers is a drawback that hinders its wide use. The development of virus-neutralizing hyperimmune globulins produced in horses or llamas may be an approach to treat infection against this SARS-CoV-2. The use of llamas⁷ for passive immunization is still experimental and limited by animal availability. On the other hand, hyperimmune serum, immunoglobulins or IgG fragments produced in equines have been used to treat many diseases such as rabies, tetanus and snake venoms, among others^{8,9}.

Brazil has a large installed capacity to produce equine hyperimmune globulin preparations for rabies, tetanus and several venoms, which makes the production of such products against SARS-CoV-2 highly feasible. Previous works with other related betacoronaviruses reported that equine hyperimmune sera resulted in neutralizing antibodies against SARS-CoV¹⁰ and MERS-CoV¹¹. In these works, the immunization was performed using virus or virus-like particles, using complete Freund's adjuvant (CFA) in the first immunization and incomplete Freund's adjuvant (IFA) in the subsequent immunizations. More

recently, the recombinant receptor-binding domain (RBD) of the SARS-CoV-2 S protein was shown to stimulate antibody production in mice and equines^{12,13}.

In the present work, we also used a recombinant antigen to immunize horses. However, the antigen chosen was the trimeric version of the complete ectodomain of the spike protein, comprising both the S1 (responsible for receptor binding) and the S2 (responsible for fusion to the cell membrane) subunits. Moreover, the chosen antigen was produced in house using in a gene construct that yields the protein in a stabilized prefusion conformation¹⁴, with the aim of maximizing the formation of high-quality neutralizing antibodies. Our strategy may be easily reproduced in any part of the world and could rapidly be used as a therapy. Equine immunization is a well-known and easily scalable technology proven for generating high titers of neutralizing antibodies, thus showing advantages over other strategies such as using convalescent human plasma. In this study, we demonstrated the extremely high neutralizing titers obtained by means of equine immunization. The final purified F(ab')₂ preparation has an average 150-fold higher PRNT₉₀ neutralizing titer when compared with human convalescent plasma from three patients in Brazil.

Results

Immunization with trimeric S protein induces high titers of specific equine IgG

We produced the trimers of the spike protein in the prefusion conformation¹⁴ by expressing the gene construct in mammalian cells as described previously¹⁵. The quality check of the spike protein was performed by SDS-PAGE and size-exclusion chromatography (Fig. S1). Five horses were

immunized with six subcutaneous injections of S protein with an interval of one week between inoculations. No adverse effects and no animal suffering were observed for any of the five horses that received the protein injections. Anti-spike IgG measured by ELISA in weekly samples showed that one week after the first immunization, anti-SARS-CoV-2 IgG was not yet detectable, but that IgG titers increased progressively after the successive immunizations (Fig. 1A). Four out of the five immunized horses produced similar amounts of specific antibodies, and one did not show a strong response (Fig. 2). In spite of the low-responding horse (#835), the average IgG titer for all five horses reached 1,180,980 after 42 days, i.e., one week after the sixth immunization (Fig. 1B), indicating that the trimeric S protein is a good immunogen to be used to induce production of specific anti-SARS-CoV-2 antibodies.

Equine sera and F(ab')₂ fragment developed against trimeric S protein have potent neutralizing titers against SARS-CoV-2

We first evaluated the *in vitro* neutralizing activity against SARS-CoV-2 in equine sera collected one week after three to six immunizations (days 28 to 42 after first immunization). PRNT₅₀ titers (average values for all five horses) seemed to achieve a plateau of approximately 1:23,000 after the fourth immunization, whereas the more stringent PRNT₉₀ titers (average values for all five horses) still showed an ascendant trend along time, reaching an average PRNT₉₀ titer of 1:14,604 on day 49. Due to the low-responding horse (#835), standard deviations are high (Fig. 3; Table S1).

Plasma from all five horses was then pooled, digested with pepsin to eliminate the Fc portion and precipitated with ammonium sulfate for purification,

resulting in a concentrate of F(ab')₂ fragments with 90 mg/mL total protein concentration. The F(ab')₂ concentrate maintained the capacity to recognize the trimeric S protein, displaying an ELISA titer of 1:1,000,000 (Fig. 4). F(ab')₂ neutralizing titers were also very high, achieving a PRNT₅₀ of 1:32,000 and a PRNT₉₀ of 1:16,000 (Fig. 3 and Table S1). We further compared the neutralizing titers of the equine samples to the neutralizing titers determined for plasma from three convalescent COVID-19 patients. Interestingly, the average neutralizing titers of equine serum (from day 49) were 78- and 138-fold higher than the average human convalescent plasma titers in terms of PRNT₅₀ and PRNT₉₀, respectively. Regarding the F(ab')₂ concentrate, the neutralizing titers were 107-fold (for PRNT₅₀) and 151-fold (for PRNT₉₀) higher than the average human convalescent plasma titer. These data show the great potential of using equine immunoglobulins in the treatment of COVID-19.

Discussion

In the setting of a pandemic, when no vaccines and no specific treatments are available, passive immunotherapies using convalescent human plasma or animal-derived hyperimmune globulins usually represent the first specific antiviral therapies to become available. In previous outbreaks caused by other viruses, such as SARS-CoV, MERS-CoV, Ebola and avian influenza virus, horse immunization to produce hyperimmune globulins was evaluated^{10,11,16,17}. Besides emerging and re-emerging infectious diseases, heterologous polyclonal antibody therapies are also useful to treat longstanding neglected tropical diseases¹⁸. Equine antivenom products are routinely produced in both high- and low-income countries¹⁶, and WHO standardized

guidelines are available for this class of products (www.who.int/bloodproducts/snake_antivenoms/snakeantivenomguide/en), potentially allowing equine antibody products to be largely available worldwide within a relatively short period of time.

Motivated by the SARS-CoV outbreak in Asia in 2002-2003, equine F(ab')₂ were developed by immunizing horses 4 times using whole virus (F69 strain propagated in Vero cells) and complete/incomplete Freund's adjuvants, resulting in ELISA titers of 1:14,210 and neutralization titers of 1:14,240¹⁰. In the case of MERS-CoV, equine immunoglobulin products were generated by immunizing horses with virus-like particles (VLPs) formed by three viral structural proteins (spike, membrane and nucleocapsid proteins). The equine antibodies were then purified by immunoaffinity chromatography, by using a resin containing the receptor binding domain of the S protein as affinity ligand¹¹. The purified anti-MERS antibody preparation presented ELISA titers of 1:20,480 and neutralization titers of 1:20,900. The binding and neutralizing titers obtained in the present work are quite superior (1:1,000,000 and 1:23,219, respectively) and indicate a high immunogenicity of the trimeric S protein used herein.

Recently, two reports have described the production of equine antibodies by immunizing horses with the recombinant SARS-CoV-2 receptor binding domain^{12,13}, which comprises approximately 16% of the spike protomer. Pan et al. evaluated in 4 horses a 4-dose immunization scheme, totalizing 33 mg of RBD injected per horse over 28 days since the first immunization¹², whereas Zylberman et al. evaluated in 2 horses a total of 3.5 mg of RBD per horse, also for 4 immunizations over 28 days¹³. Both works reached titers of binding antibodies in the range of 1:1,000,000, thus similar to the titers obtained in the

present work. Zylberman et al. apparently achieved complete neutralization at a 1:10,240 dilution¹³, whereas Pan et al. reported 80% neutralization for a 1:2,560 dilution¹². In the present work, although our immunization strategy used only 1.2 mg of S protein per each of the 5 horses (6 weekly doses of only 200 µg), we achieved binding titers over 1:1,000,000 (Fig. 1B) and, more importantly, average titers for 90% neutralization were 1:14,604 if the low responding horse is included, or 1:18,000 if the low-responding animal is not included (Table S1). The higher ratio of neutralizing to binding antibodies obtained in the present work indicates a high quality of the antibodies generated by using the prefusion trimeric spike protein as immunogen. Our results are in agreement with a previous comparison of mice immunization with the trimeric spike protein and the RBD, which showed higher neutralizing titers for the S protein¹⁹. This is probably related to the fact that other domains of the spike protein besides the receptor binding domain are also targets for neutralizing antibodies²⁰. Not surprisingly, most COVID-19 vaccines under development worldwide focus on the spike protein, either as the recombinant protein itself, or as nucleic acid encoding the S protein, such as in the case of mRNA or vectored vaccines.

In our study, we used Montanide ISA 50V as adjuvant. In previous studies, horse hyperimmunization against SARS-CoV¹⁰, MERS-CoV¹¹ and SARS-CoV-2^{12,13} was performed using complete/incomplete Freund's adjuvant (CFA/IFA). Montanide adjuvant enhances the immune response by warranting depot effect and promoting slow delivery of the protein, in order to maintain recognition, stimulation and phagocytosis by dendritic cells and B cells for long times²¹. The present results not only indicate the high immunogenicity of the

trimeric prefusion spike protein but also demonstrate the feasibility of using Montanide, which is a less reactogenic adjuvant than CFA/IFA²².

In order to avoid the risk of antibody-dependent enhancement (ADE) of infection, the pooled plasma obtained from all 5 horses 49 days after the first immunization was processed to remove the Fc portion of IgG by pepsin digestion, followed by a partial purification of the F(ab')₂ fragment by ammonium sulfate fractionation. This is a proven technology used worldwide for the production of equine hyperimmune products, since the use of F(ab')₂ instead of whole IgG eliminates non-specific binding between the Fc portion of antibodies and Fc receptors on the cells, thus avoiding ADE. This technology is regularly used for GMP manufacture of anti-rabies, anti-tetanus and anti-venom F(ab')₂ products at Vital Brazil Institute, with no records of hypersensitivity issues and decades of excellent safety track record. Also, safety trials of equine F(ab')₂ against avian influenza (H5N1) performed by Bal et al. confirmed that it is a well-tolerated product, causing just a few mild adverse events¹⁷. Importantly, the anti-SARS-CoV-2 F(ab')₂ concentrate developed herein maintained the very high binding and neutralizing titers found in unprocessed sera (Figs. 3 and 4).

As aforementioned, convalescent plasma is another therapeutic alternative that quickly becomes available in the setting of outbreaks. For comparison, we evaluated the neutralization titer of three human convalescent plasma samples. We found that the neutralization titers of equine F(ab')₂ concentrate were two orders of magnitude higher than those of human convalescent plasma (107- and 151-fold higher for PRNT₅₀ and PRNT₉₀, respectively). Plasma from the low-responding horse #835, which started to show relevant neutralizing activities just after the fourth inoculation, was

included in the plasma pool used to produce the F(ab')₂ concentrate. Thus, if horses would be pre-selected for their neutralizing activity prior to F(ab')₂ manufacture, the neutralizing ability of the antibody concentrate could be even higher, reducing infusion volumes in patients. Zylberman et al., who used SARS-CoV-2 RBD to immunize horses, reported their F(ab')₂ product to have an approximately 50-fold higher neutralizing capacity than convalescent plasma reported in literature¹³. The other work using the RBD to immunize horses evaluated samples from 11 COVID-19 convalescent patients and observed that at a 1:640 dilution three human samples showed 50% neutralization and another two showed 80-90% neutralization ability. In contrast, their unprocessed horse antisera after three immunizations showed complete neutralization at 1:640 dilution, and 50-60% neutralization at 1:10,240 dilution¹². As a whole, these data confirm the promising potential of equine hyperimmune products as COVID-19 countermeasure over human convalescent plasma, considering the high potency and safety record of F(ab')₂ products, as well as eventual limitations related to human plasma availability and eventual risks of adventitious agents transmission of human plasma products.

The advantage of using the fully folded trimeric S protein in the prefusion conformation to elicit equine polyclonal antibodies against SARS-CoV-2 is the main novelty in our work. In a recent report, Liu et al. reported that highly neutralizing monoclonal antibodies discovered from B cells of convalescent COVID-19 patients targeted epitopes in the receptor-binding domain (RBD) and in the N-terminal domain (NTD), as well as in quaternary epitopes of the trimeric spike protein²⁰. Thus, the use of the trimeric spike protein combines the advantages of higher immunogenicity as compared to smaller protein fragments

(such as the RBD used in other works^{12,13}) with low biosafety concerns as compared to the inoculation of the whole virus (as used for SARS-CoV)¹⁰. Figure 5 illustrates our whole strategy and emphasizes the potential binding of F(ab')₂ fragments to the S protein on the viral surface.

In summary, the use of an anti-COVID-19 hyperimmune F(ab')₂ concentrate produced by using a potent antigen (the prefusion trimeric spike glycoprotein) is a prompt alternative for passive immunization therapy, especially in a situation where no vaccine has been approved (Fig. 5). Even in a better scenario of an available efficacious vaccine, the possibility of having equine antibody products is highly important as it has been proved in the case of rabies vaccine, where both passive and active immunizations are used to save lives. Hyperimmune F(ab')₂ concentrates are manufactured in existing facilities available worldwide, both in high- and low-income countries, using a long proven platform technology, which could help accelerating the regulatory pathway up to product approval for human use.

Taking as reference the production of other registered (Fab')₂ concentrates regularly produced by Vital Brazil Institute in Brazil (e.g. anti-rabies), each horse can supply 16 L of blood per bleeding, which yields approximately 10 L of plasma for processing and results in at least 200 ampoules of F(ab')₂ concentrate. Since one horse can be bled up to six times a year without any animal suffering, this would mean that producing 100,000 ampoules (doses) per year would require approximately 80 horses per year. This is still a rough order-of-magnitude estimate, since the precise dose size still needs further studies to be defined.

Altogether, our results evidence that equine anti-COVID-19 immunoglobulin has a high chance of treating patients and contributing to change the course of COVID-19 pandemic.

Methodology

Production and purification of recombinant SARS-CoV-2 spike (S) glycoprotein

The cell line HEK293-COV2-S was generated by Alvim et al. (2020)¹⁵ and stably expresses the soluble ectodomain of the spike protein of SARS-CoV-2 in the prefusion trimeric conformation¹⁴. The cells were cultivated in HEK-GM medium (Xell AG, Germany), either at 300-mL scale at 37°C and 5% CO₂ in erlenmeyer flasks under orbital agitation (180 rpm, 5-cm stroke) or in 1.5-L stirred-tank bioreactors operated at a pH setpoint of 7.1 and a dissolved oxygen setpoint of 40% of air saturation. Cell-free supernatant was obtained by microfiltration with 0.45- μ m PVDF membranes and injected in a 5-mL StrepTrap XT affinity chromatography column (Cytiva) following manufacturer's instructions. Protein concentration, purity and identity in the eluted fractions were confirmed by Nanodrop (ThermoFisher), SDS-PAGE and Western blot analyses, respectively. The purified protein obtained in the affinity chromatography eluate was used to immunize horses and as antigen in the ELISA assay to detect anti-SARS-CoV-2 antibodies in samples.

Animal immunization

All the procedures involving animals were in accordance with the animal research ethical principles determined by the National Brazilian Law 11.794/08.

The protocol was approved by the Animal Care and Use Committee from Vital Brazil Institute (IVB) under permission no. 003.

All animals were subjected to prophylactic vaccination and deworming programs routinely utilized at the IVB farm and were tested for equine infectious anemia and *Burkholderia mallei*, as determined by the Brazilian Ministry of Agriculture regulations.

Five 3-5 year-old healthy horses (3 males and 2 females) from the IVB farm, weighing approximately 350 kg each, were used for the production of polyvalent sera. Before immunization, blood samples were drawn from the jugular vein and sera was stored at -20°C for use as negative controls in the binding and neutralizing antibody determinations. Each horse was subcutaneously immunized six times at 7-day intervals (on days 0, 7, 14, 21, 28 and 35) in different positions of the dorsal region. Each immunization of each horse consisted of 200 µg recombinant SARS-CoV-2 trimeric S protein mixed with Montanide ISA 50V adjuvant (Seppic, France) to form an emulsion (one part of immunogen in sterile saline to one part of sterile adjuvant). The horses were checked daily, and food and water intakes were monitored. Blood samples were collected every 7 days just before the next inoculation, and 7 and 14 days after the last inoculation (up to day 49 since the first inoculation). Sera were stored at -20°C for the measurement of binding and neutralizing antibody titers.

Production of a bench-scale F(ab')₂ lot

Plasma processing to produce F(ab')₂ was initiated by adding 3 L of water and 15 mL of 90% phenol solution to 2 L of horse plasma in a reactor. The solution was homogenized for 10 min and the pH was adjusted to 4.3. Under agitation,

1.25 g/L of pepsin was added, pH was adjusted to 3.2 and the sample remained under agitation for 10 min. The sample was then stirred at 37°C and pH was adjusted to 4.2 with sodium hydroxide. Under constant agitation, sodium pyrophosphate decahydrate (12.6 mM) and toluene (10 µM) were added. Later, ammonium sulfate was added at 12% (m/v) and the solution was incubated at 55°C for 1 h.

To separate the F(ab')₂ fragments, the Fc portion was precipitated and subsequently filtered under pressure at constant agitation. The F(ab')₂ was recovered from the liquid phase. To subsequently precipitate F(ab')₂ from the liquid phase, ammonium sulfate was added at 19% (m/v), and a second precipitation was performed under constant agitation and alkaline pH. Subsequently, the solution was diafiltered using a 30 kDa tangential ultrafiltration system until ammonium sulfate became undetectable in the retentate. The samples were isotonized with 15 mM NaCl and 90% phenol solution was added to a final concentration of 0.3% (v/v). After sterile filtration, the F(ab')₂ product was stored at 4°C.

Enzyme-linked immunosorbent assay (ELISA)

In brief, polystyrene high-adsorption 96-well microplates (ThermoFisher, USA) were coated with 500 ng/well of recombinant SARS-CoV-2 S protein (100 µL/well at 5 µg/mL) in carbonate-bicarbonate buffer (pH 9.6) overnight at room temperature and then blocked with 3% BSA (Sigma, USA) in PBS for 2 h at 37°C. Serially diluted serum samples of the 5 horses were added to the plate and incubated at 37°C for 1 h. Horseradish peroxidase-conjugated rabbit anti-

horse IgG (Sigma A6917, USA) diluted 1:10,000 in PBS was incubated at 37 °C for 1 h. After each step, the plates were washed 3 times with PBST (PBS containing 0.05% Tween 20). OPD substrate (100 µL/well - Sigma, USA) was added to wells and incubated in the dark for 10 min at room temperature. The reaction was stopped by adding 50 µL of 30% H₂SO₄ (v/v) to each well, and the absorbance value was measured at 490 nm in a microplate reader (Epoch/2 microplate Teaser, Biotek). IgG antibody titer was defined as the highest dilution of serum yielding an absorbance ratio greater than 2, in the same dilution (λ_{490} of sample/ λ_{490} of negative control). The analyses were carried out in duplicate.

Cells and virus used in neutralization assays

African green monkey kidney (Vero, subtype E6) cells were cultured at 37°C in high glucose DMEM with 10% fetal bovine serum (Hyclone/Cytiva, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (ThermoFisher, USA) in a humidified atmosphere with 5% CO₂.

SARS-CoV-2 was prepared in Vero E6 cells. Originally, the isolate was obtained from a nasopharyngeal swab from a confirmed case in Rio de Janeiro, Brazil (IRB approval 30650420.4.1001.0008). All procedures related to virus culture were handled in a biosafety level 3 (BSL3) multiuser facility according to WHO guidelines. Virus titers were determined as plaque forming units (PFU)/mL. The virus strain was sequenced to confirm the identity and complete genome is available in Genbank (SARS-CoV-2/human/BRA/RJ01/2020, #MT710714). The virus stocks were kept at - 80°C.

Neutralization assay

To access the neutralization titer the samples were incubated with 100 plaque forming units of SARS-CoV-2 for 1 h at 37°C. Then, the samples transferred to 96-well plates with monolayers of Vero cells (2×10^4 cells/well) with serial dilutions of sample for 1 h at 37°C. Cells were washed, and fresh medium with 2% FBS and 2.4% CMC was added. On day 3 post-infection the cytopathic effect was scored in at least 2 replicates per dilution by independent readers. The reader was blind with respect to the sample ID.

Statistical analyses were performed with GraphPad Prism 7®. Neutralization assay data are shown as mean \pm SD; $p < 0.05$ vs plasma collected on day 0 according to Student's t-test for paired samples.

Sample collection from human subjects

Samples collected at the State Hematology Institute Hemorio followed a protocol approved by the local ethics committee (CEP Hemorio; approval #4008095) as described previously¹⁵.

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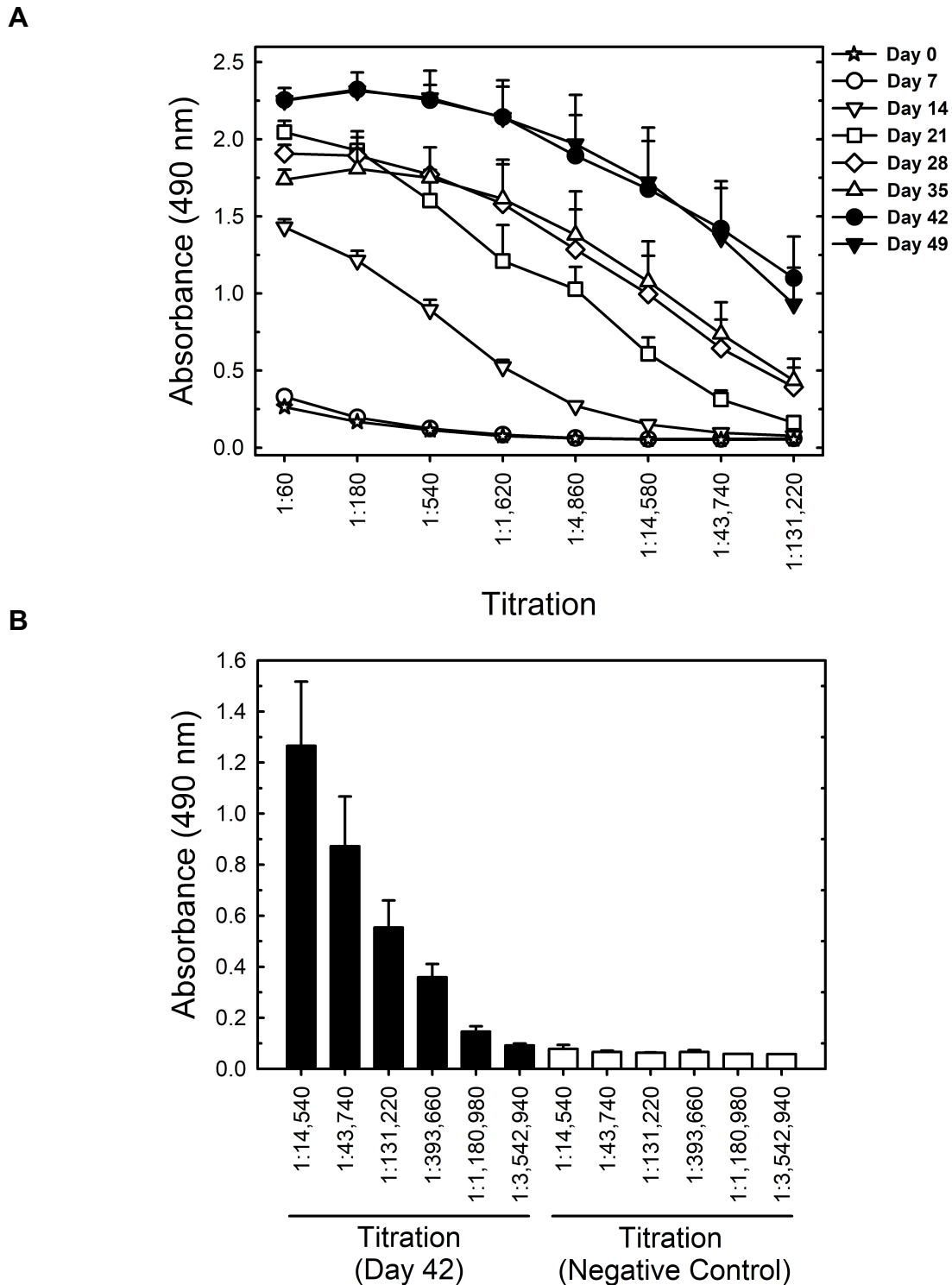


FIGURE 1. Titration of horse antibodies by S-protein ELISA. (A) Titration up to 131,200-fold dilution of serum samples collected from immunized horses on different days after first immunization. **(B)** Titration up to 3,542,940-fold dilution of samples collected on day 42 after first immunization. All results shown are mean \pm standard error for all 5 horses. The negative control is a pool of pre-immune sera collected from all 5 horses.

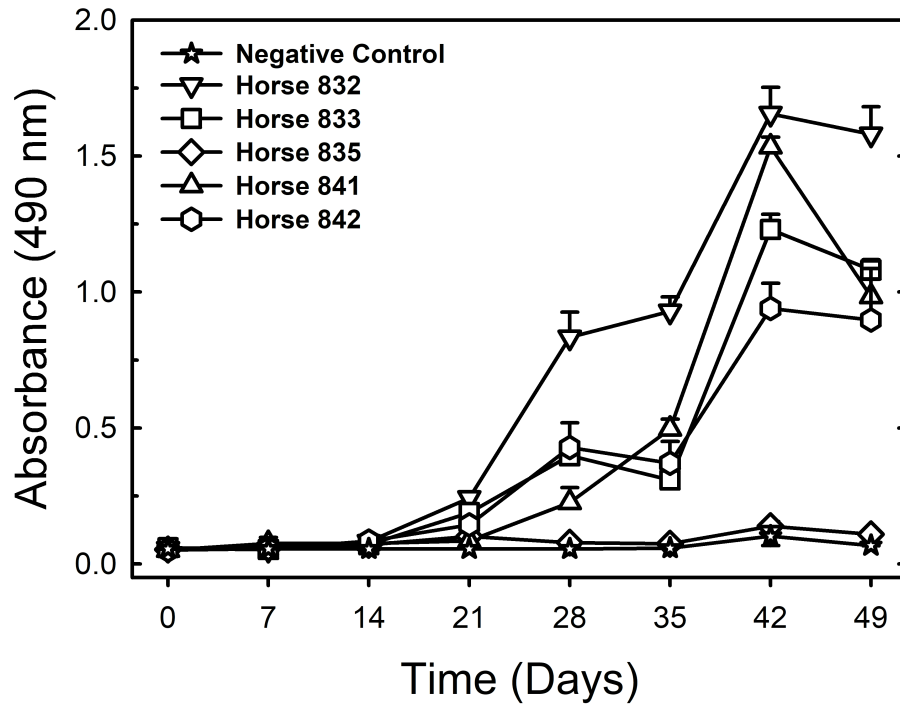


FIGURE 2. Anti-S antibodies measured by ELISA in horse serum samples collected along time. All sera were diluted 1:131,220. The results are shown as mean \pm standard error for analytical duplicates. The negative control is a pool of pre-immune sera collected from all 5 horses.

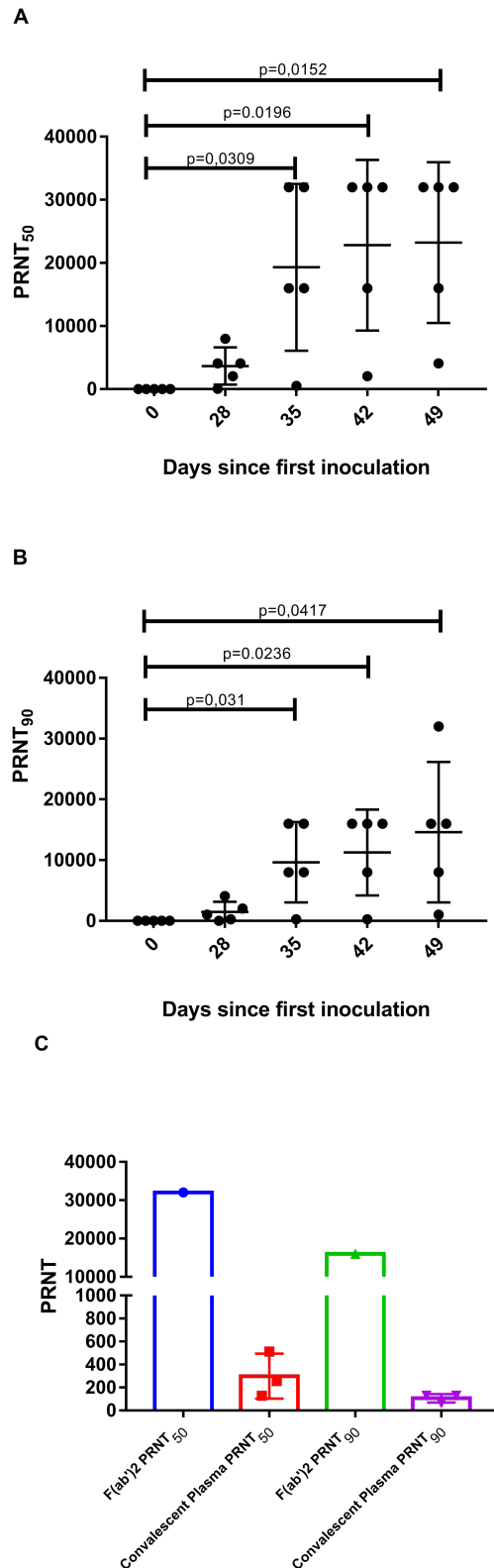


FIGURE 3. Microneutralization assays. (A) PRNT₅₀ and (B) PRNT₉₀ for equine plasma collected on days 28 to 49 after first immunization. Data shown as mean \pm SD; $p < 0.05$ vs plasma collected on day 0 according to t-test for paired samples. (C) Comparison of PRNT₅₀ and PRNT₉₀ for equine F(ab')₂ concentrate and human convalescent plasma. Data shown as mean \pm standard deviation.

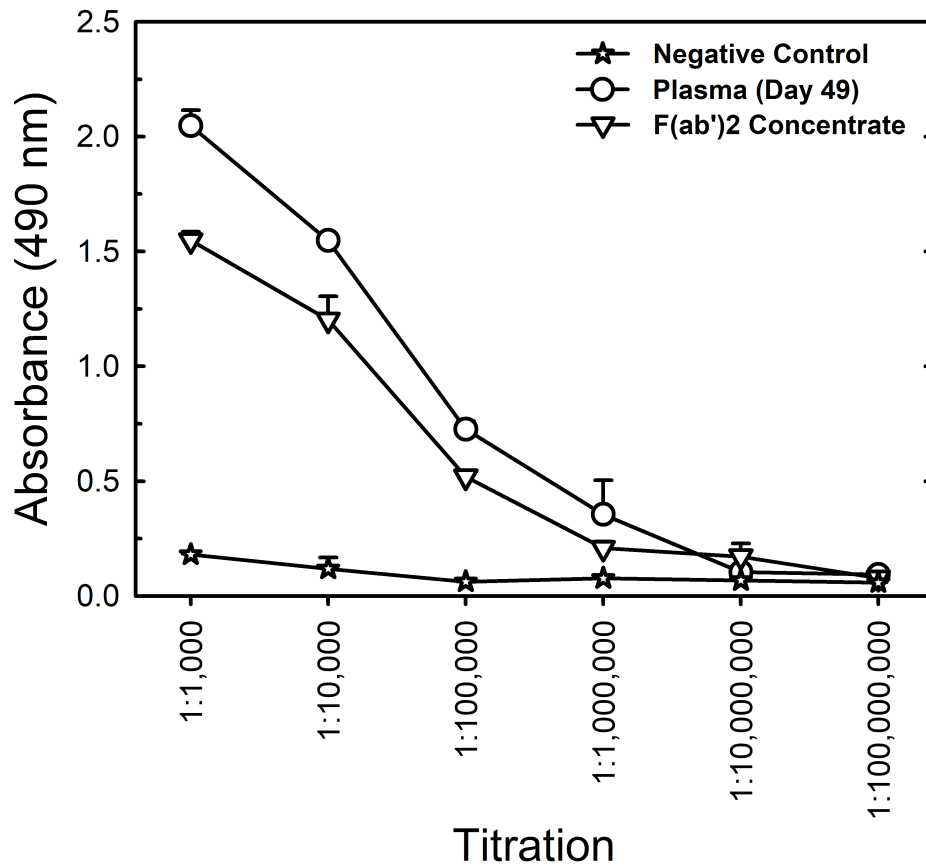


FIGURE 4. Comparison of S-protein ELISA titration of equine F(ab')₂ concentrate and horse plasma collected on day 49 after first immunization. The sample from day 49 and the pre-immune negative control are pools of samples collected from the 5 horses. The results are shown as mean ± standard error for analytical duplicates.

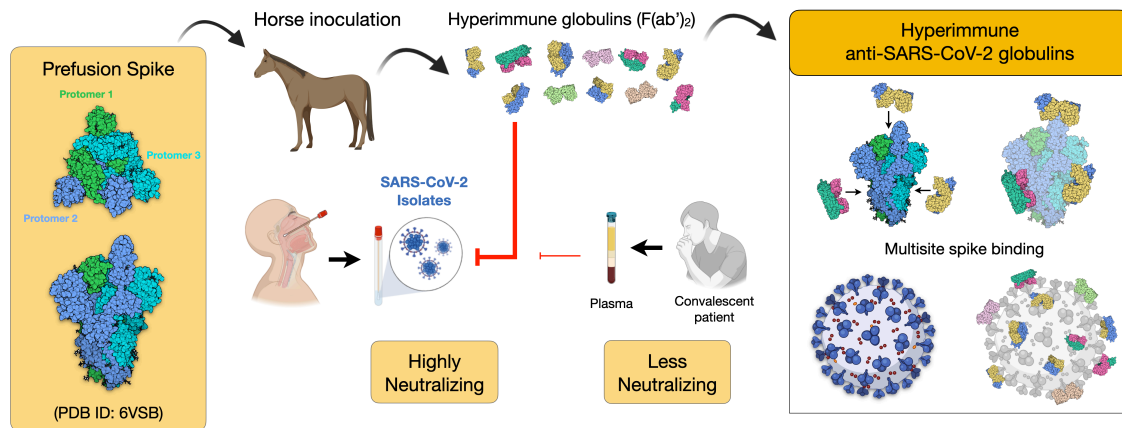


FIGURE 5. Scheme of the immunization strategy and anti-SARS-CoV-2 hyperimmune globulin production. Recombinant prefusion trimeric S protein is used to inoculate horses and to produce hyperimmune F(ab')₂ concentrate. The equine antibody preparation presented a much higher capacity to neutralize a SARS-CoV-2 isolate than human convalescent plasma. One advantage of using the full-length recombinant spike trimer is the production of antibodies against different antigenic segments of the viral protein. This strategy may result in more efficient neutralizing capacity than antibodies produced against isolated fragments of the spike protein, such as the receptor binding domain (RBD).

SUPPLEMENTARY MATERIAL

Table S1. Microneutralization data for equine sera collected along the immunization process, for F(ab')₂ concentrate and for human convalescent plasma.

Sample ID		PRNT ₅₀	Mean±SD	PRNT ₉₀	Mean±SD
Equine pre-immune serum	832	<16	16±0	<16	16±0
	833	<16		<16	
	835	<16		<16	
	841	<16		<16	
	842	<16		<16	
Equine serum 28 days	832	>8000	3660±2947	>4096	1491±1655
	833	>4096		>2048	
	835	>64		>32	
	841	>2048		>256	
	842	>4096		>1024	
Equine serum 35 days	832	>16000	19302±13203	>8000	9651±6601
	833	>32000		>16000	
	835	>512		>256	
	841	>32000		>16000	
	842	>16000		>8000	
Equine serum 42 days	832	>>32000	22809±13516	>16000	11251±7055
	833	>16000		>8000	
	835	>2048		>256	
	841	>32000		>16000	
	842	>32000		>16000	
Equine serum 49 days	832	>32000	23219±12738	>32000	14604±11560
	833	>16000		>8000	
	835	>4096		>1024	
	841	>32000		>16000	
	842	>32000		>16000	
Equine F(ab')₂ concentrate	Pilot lot	>32000		>16000	
Human convalescent plasma	1	>512	298±195	>128	106±36
	2	>128		>64	
	3	>256		>128	

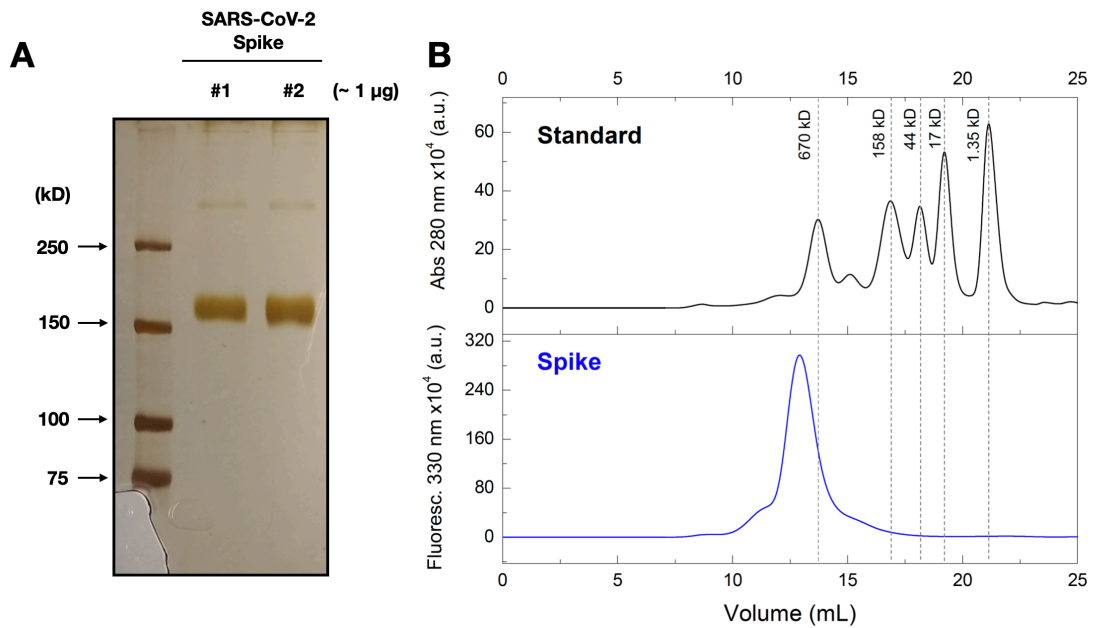


Figure S1. Quality checks of SARS-CoV-2 spike protein. a, Image of a silver stained 7% SDS-PAGE showing two representative batches of the SARS-CoV-2 spike prep (two batches for purposes of comparison). b, Line plots showing size exclusion chromatograms from a Superose 6 10/300 column. The fluorescence intensity at 330 nm of the spike protein was recorded as a function of the retention volume. Absorbance at 280 nm of a molecular weight standard is shown in black lines for comparison.