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BRIEF REPORT

Potent Neutralizing Activity of Polyclonal Equine Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 Variants of Concern

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Several anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) monoclonal antibodies (mAbs) have received emergency authorization for coronavirus disease 2019 (COVID-19) treatment. However, most of these mAbs are not active against the highly mutated Omicron SARS-CoV-2 subvariants. We have tested a polyclonal approach of equine anti-SARS-CoV-2 F(ab')2 antibodies that achieved a high level of neutralizing potency against all SARS-CoV-2 variants of concern tested including Omicron BA.1, BA.2, BA.2.12 and BA.4/5. A repertoire of antibodies targeting conserved epitopes in different regions of the spike protein could plausibly account for this remarkable breadth of neutralization. These results warrant the clinical investigation of equine polyclonal F(ab')2 antibodies as a novel therapeutic strategy against COVID-19.

Keywords. COVID-19; SARS-CoV-2; neutralizing antibodies; Omicron; variants of concern.

The rapid widespread of severe acute respiratory syndrome (SARS) coronavirus (CoV) 2 (SARS-CoV-2) during the coronavirus disease 2019 (COVID-19) pandemic has caused 550 million confirmed cases and >6 million deaths by July 2022, along with significant socioeconomic disruptions. This active transmission of SARS-CoV-2 has resulted in the emergence of several variants of concern (VoC) that have apparently been selected by a higher transmissibility and have challenged the public health control strategies to contain the pandemic. The recent appearance of the Omicron VoC, which combines an augmented transmission capability along with evasion from neutralization by convalescent or vaccinee serum, is a further hurdle for pandemic control [1]. A number of potent monoclonal antibodies (mAbs) have received emergency authorization from European Medicines Agency and the Food and Drug Administration for COVID-19 treatment in selected patients. However, most of these mAbs have been found to be inefficacious against the highly mutated Omicron variant. The emergence of Omicron subvariants BA.1, BA.2, BA.2.12, BA.4 and BA.5, with different sensitivity to mAbs, has complicated the scenario of immune therapeutics [2].

Considering the experience on the evolution of SARS-CoV-2 into a diversity of variants, a polyclonal approach in which many potential epitopes within the SARS-CoV-2 Spike protein are targeted could have great advantages in terms of breadth to neutralize current and potential future SARS-CoV-2 VoC. On the other hand, heterologous immunoglobulins have been used for more than a century in human therapy, especially for envenomation, rabies, and tetanus [3].

Specific polyclonal immunoglobulins are indeed a well-known and effective therapeutic alternative that can be used quickly to respond against major health risks such as pandemics, emerging diseases, and bioterrorism. FBR-002 is composed of purified polyclonal equine fragments F(ab')2 recognizing SARS-CoV-2 spike protein. FBR-002 represents a serious therapy candidate for treating patients hospitalized with COVID-19 because of (1) its polyclonality, which allows targeting of multiple epitopes of the spike protein, limiting the risk of viral escape if new strains emerge, and (2) its highly purifying process and lack of Fc portion, minimizing the risk of antibody-dependent enhancement, immunogenicity, and overall adverse effects pattern (eg, serum sickness syndrome) compared with whole immunoglobulins that may be used for passive immunotherapy. Therefore, F(ab')2 polyclonal fragments show fewer safety concerns than other related products, such as plasma from recovered patients or polyclonal humanized anti-SARS-CoV2 antibodies [3].

A retrospective analysis of Favirab, anti-rabies F(ab')2 fragments produced with similar technology than FBR-002, covering an approximation of 650 000 to 1 million treated patients found a very low rate (0.18%) of immediate hypersensitivity and supports the general safety of the compound [4]. Many other supportive safety data are available on other equine F(ab')2 products, suggesting a strong historic of clinical use and pharmacovigilance on such highly purified product [5, 6].

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In this report, we describe the in vitro evaluation of FBR-002 against SARS-CoV-2 VoC, a clinical grade product of polyclonal F(ab')2 immunoglobulin fragments from Fab'entech.

METHODS

Equine hyperimmune plasma: Three healthy French trotter horses were hyperimmunized with full SARS-CoV-2 spike protein. Horses had no detectable antibodies against SARS-CoV-2 before immunization and were strictly controlled for several viruses. Blood samples were collected regularly after immunization and plasma were prepared and stored at –20°C.

For preparation of F(ab')2 fragments, pooled horse plasma was purified to obtain highly purified F(ab')2 fragment, as described elsewhere [7, 8]. The main purification steps consist of (1) anion-exchange chromatography steps, which eliminate proteins including albumin; (2) hydrolysis of whole immunoglobulins into F(ab')2 fragments in order to eliminate Fc fragments; and (3) pasteurization (heat treatment) at 60°C for 10 hours to ensure viral safety of the product. The final bulk product (FBR-002) is filtered at a level of 0.2 µm and then stored at +5°C ± 3°C until use. The FBR-002 batch used in this study was purified (>99.5%) under good manufacturing practice conditions and prepared at a final concentration of 44.81 g/L.

Neutralization assays using the rVSV-luc pseudovirus system (PSV) were performed following a previously published protocol [9] against SARS-CoV-2 Wu-hu-1 S-D614G as a reference; VoC Alpha, Beta, Gamma, Delta, Omicron BA.1, BA.2, BA.2.12.1, and BA.4/5; variants Mu, Kappa, Iota, and Epsilon; and Middle East respiratory syndrome (MERS) CoV, SARS-CoV-1, and Ebola virus (EBOV) strain Makona GP. Neutralization potency was calibrated using the World Health Organization international standard 20/136 and the well-characterized mAb Sotrovimab [6]. Full details and sequence references are provided in the Supplementary Methods. For the microneutralization assay with authentic SARS, neutralization against the reference virus SARS-CoV-2 D614G and the VoC Delta, BA.1, and BA.4 was determined according to protocols described elsewhere [10] and in the Supplementary Methods.

RESULTS

Results of the neutralization assays with PSV and authentic SARS-CoV-2 against representative VoC and controls are shown in Figure 1*A* and 1*B,* respectively. The 50% neutralization titers for the complete set of VoC and controls are summarized in Table 1. FBR-002 achieved a very high level of neutralizing potency against all SARS-CoV-2 VoC tested: in the PSV assay, the neutralization titer was 546 827 IU/mL for the reference spike sequence (614G) and in the range of 0.5–6×10^5 IU/mL for the other VoC. Specifically, neutralizing titers against the Omicron VoC BA.1, BA.2, BA.2.12.1, and BA.4/5 were 10 7355, 12 7229, 87 193, and 65 082 IU/mL, respectively (Table 1). The microneutralization assay with authentic SARS-CoV-2 yielded quite similar levels of neutralization with selected VoC using FBR-002 (Figure 1*B*, Table 1, and Supplementary Figure 1). Based on the immunoglobulin concentration of FBR-002 (4481 g/L) the neutralizing titer of 511 920 IU/mL obtained in the PSV assay against the reference SARS-CoV-2 S-D614G would mean a median inhibitory concentration (IC_{50}) of 81.9 ng/mL, and subsequently the IC_{50} for BA.4/5 is 688.5 ng/mL.

Although the activity was much lower, we observed FBR-002 neutralization against a SARS-CoV-1 PSV, with a titer of 13 232 IU/mL. No measurable neutralizing activity was detected against MERS, Ebola virus, or vesicular stomatitis virus.

DISCUSSION

The emergence of diversity along the evolution of the COVID-19 pandemic has led to the emergence of different SARS-CoV-2 variants whose adaptation properties to human transmission have resulted in an increased capability for transmission and in immune evasion to the neutralizing antibody response elicited by infection and or vaccination that is challenging the control of the pandemic. Among the antiviral strategies displayed, a number of mAbs have been developed for therapeutic purposes. mAbs have been shown to be clinically effective for treating severe cases of emergent pathogens, such as Ebola virus, and have been actively sought after for COVID-19.

Most of the cloned neutralizing antibodies recognize epitopes within the receptor-binding motif, the contact region of the receptor-binding domain with the angiotensin-converting enzyme 2 receptor. These antibodies, which recognize residues within the receptor-binding motif, are among the most common produced in response to SARS-CoV-2 infection in humans. However, the evolution of scape mutations in the circulating VoC have rendered some of them inefficacious [11]. This capability for immune evasion has been exhibited to a new limit by the recent emergence of the Omicron VoC, which is resistant to neutralization by most of the clinically available mAbs.

Using a polyclonal approach with equine neutralizing anti-SARS-CoV-2 polyclonal F(ab')2 antibodies, we have achieved extraordinary neutralizing potency that is 2–3 orders of magnitude what is normally achieved in response to SARS-CoV-2 infection and/or vaccination [12]. The neutralization coverage for the different variants is also remarkable, reaching $>10^5$ IU/mL for most VoC tested. As expected, Omicron showed the highest reduction in neutralization: 5.1-, 4.3-, 6.3-, and 8.4-fold for BA.1, BA.2, BA.2.12.1, and

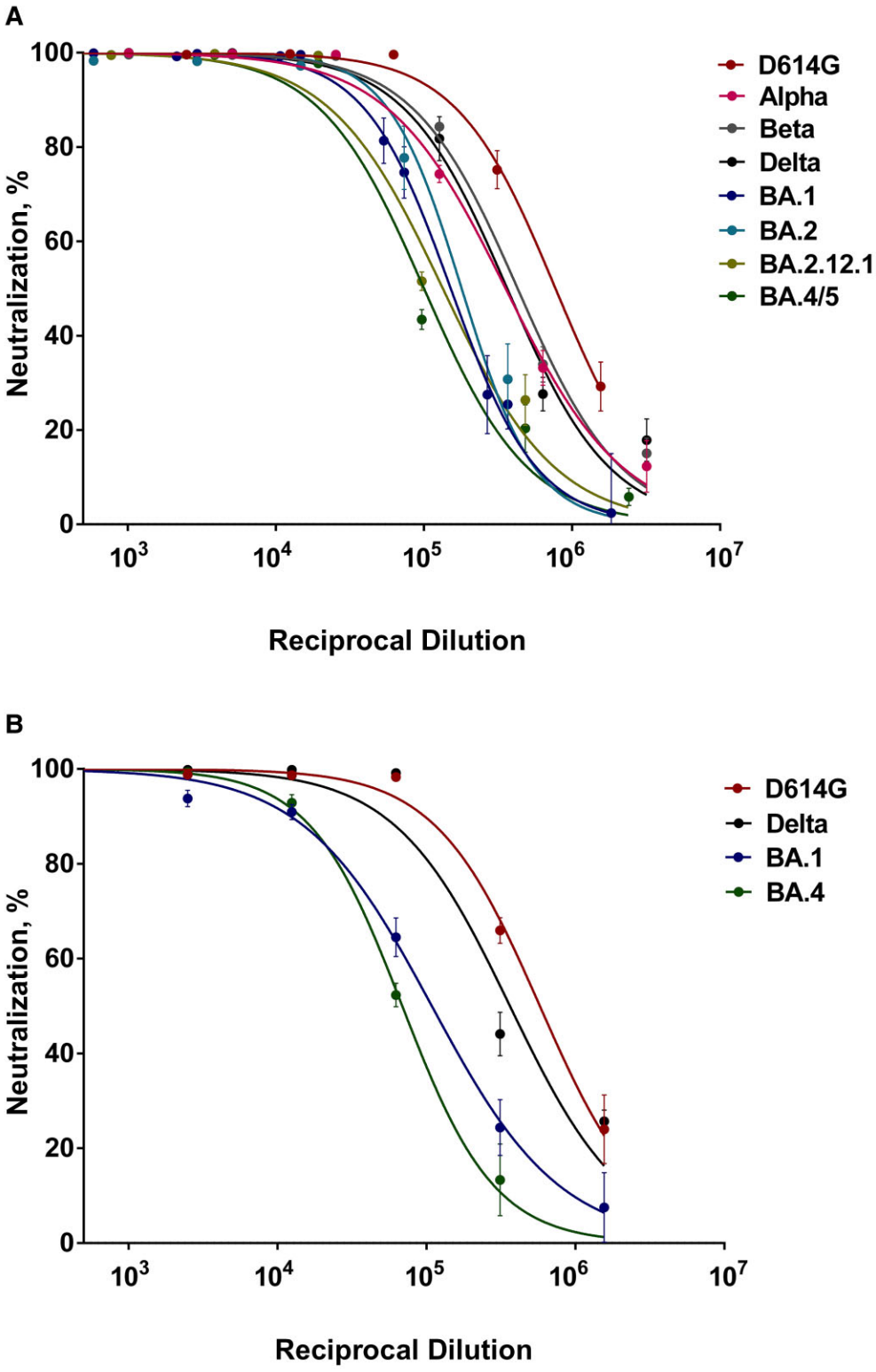

Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing activity curves for FBR-002. *A*, SARS-CoV-2 variant of concern pseudotyped rVSV-luc: reference D614G, Alpha, Beta, Delta, BA.1, BA.2, BA.2.12.1, and BA.4/5. *B*, SARS-CoV-2 variants of concern authentic clinical isolates: reference D614G, Delta, BA.1, and BA.4. Neutralizing activity curves were calculated from individual results obtained by 3–12 replicates, using a nonlinear regression model fit with settings for log inhibitor versus normalized response curves with GraphPad Prism software (version 8).

Table 1. Neutralizing Levels for FBR-002 Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Authentic Clinical Isolates, SARS-CoV-2 Pseudotyped Particles, and Controls and Median Inhibitory Concentrations With Sotrovimab[a]

	Neutralizing Level or IC_{50}	95% CI
Neutralizing level, IU/mL		
D614G authentic	511 920	426 600–614 588
D614G	546 827	477 365–626 330
Alpha	251 961	214 938–295 359
Beta	303 479	266 704–345 477
Gamma	390 331	343 295–443 782
Delta authentic	322 278	238 006–436 481
Delta	258 224	205 199–324 977
BA.1 authentic	96 378	73 679–126 031
BA.1	107 355	91 610–125 802
BA.2	127 229	68 927–234 795
BA.2.12.1	87 193	70 155–108 368
BA.4 authentic	61 035	47 512–78 374
BA.4/5	65 082	54 567–77 589
Mu	252 592	222 100–287 195
Kappa	263 227	173 630–398 927
Iota	246 339	195 544–310 276
Epsilon	204 250	160 604–259 743
SARS-CoV-1	11 803	7563–18 418
MERS-CoV	ND	…
EBOV[b]	ND	…
VSV[b]	ND	…
IC_{50} for Sotrovimab,[c] ng/mL		
D614G authentic	582	458–738
D614G	481	362–641
BA.1 authentic	2349	1758–3139
BA.1	1717	1213–2431
BA.4 authentic	2950	1365–6376
BA.4/5	1116	8769–1419

Abbreviations: CI, confidence interval; EBOV, Ebola virus; IC_{50}, median inhibitory concentration; MERS-CoV, Middle East respiratory syndrome coronavirus; ND, not detected; SARS-CoV-1, severe acute respiratory syndrome coronavirus 1; VSV, vesicular stomatitis virus.

[a]Calibrated 50% neutralization titers were calculated according to the World Health Organization international standard 20/136, tested in parallel in each assay.

[b]EBOV and VSV were used as controls.

[c]As a control, Sotrovimab was tested in parallel against SARS-CoV-2 authentic virus D614G, BA.1, and BA.4 and SARS-CoV-2 pseudotyped RVSV-luc D614G, BA.1, and BA.4/5.

BA.4/5, respectively, with the PSV assay, compared with the ancestral D614G sequence.

Results were quite similar for Omicron BA.1 and BA.4 in the microneutralization assay with authentic SARS-Cov-2, with 5.3- and 8.4-fold reduction, respectively. This neutralizing titer against Omicron is in fact 2 orders of magnitude higher that those obtained after booster vaccination in either COVID-19–naive or COVID-19–convalescent individuals [13] and J. Luczkowiak and colleagues (personal communication). An extrapolation of neutralizing potency of FBR-002 based on its immunoglobulin content would translate to an IC_{50} of 819 ng/mL for the SARS-CoV-2 reference sequence in the PSV assay (87.5 ng/mL in the authentic virus assay) and 688 ng/mL for Omicron BA.4/5 (734.2 ng/mL for BA.4 in the authentic virus assay). This potency is significant, considering the limited availability of mAbs that are active against the Omicron subvariants.

Potency and breadth are properties of high-titer polyclonal preparation that could be particularly helpful for targeting an evolving agent such as SARS-CoV-2; considering the current level of transmission, even in highly immunized regions, the emergence of new variants can be expected. The presence of high titers of a repertoire of antibodies targeting conserved epitopes in different regions of the spike protein, such as the receptor binding domain [14] and also the N-terminal domain [15], could plausibly account for this remarkable breadth of neutralization and a more resilient activity against VoC. In this sense the neutralizing potency of the preparation is also significant against the related SARS-CoV-1 responsible for the SARS epidemic in 2002–2003. These results warrant the clinical investigation of anti-SARS-CoV-2 equine polyclonal F(ab')2 antibodies as a novel therapeutic strategy against COVID-19.

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

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Potential conflicts of interest. P. R., L. N., and C. H. H.) are employees of Fab'entech, the producer of the equine antibodies. All other authors report no potential conflicts. 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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