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RESEARCH

Analysis of specificity of COVID-19 convalescent plasma and purified antibodies against SARS-CoV-2 spike-protein linear peptides

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ABSTRACT

Knowledge about epitopes present in the SARS-CoV-2 spike protein (S-protein) is continuously increasing, to help on developing vaccines against the SARS-CoV-2 infection with specific neutralizing antibodies. COVID-19 convalescent patients' plasma (CCPP) has been used as one of therapeutic strategies to passively transfer specific neutralizing antibodies against SARS-CoV-2. However, CCPP has some drawbacks, such as availability (on time), biosafety, different specific neutralizing antibody concentration (batch-to-batch inconsistency) and quality. Hence, a mixture of safe, pure and concentrated antibody preparations specific for the S-protein was obtained, instead of CCPP. Antibodies were purified by Protein A-Sepharose affinity chromatography from five CCPP and its specificity analyzed against 23 linear peptides of the S-protein-receptor binding domain (RBD), developed through bioinformatics. The obtained antibody preparations had an average 2.43 ± 0.36 mg/mL protein concentration, and 96.10 ± 0.89 and 95.6 ± 1.29 % purity, as determined under reducing and non-reducing conditions, respectively. The peptide recognition analysis evidenced that CCPP recognized only 13 out of the 23 linear peptides, while the purified antibody preparations recognized all of them. This corroborated the diversity of the B-cell response and the interference by some CCPP components in ELISA performance. As conclusions, some components of CCPP may not affect the ELISA sensitivity, which did not recognize ten RBD-linear peptides. The IgG-type antibodies' amounts in the CCPP remained unaffected after the protein A-Sepharose affinity chromatography purification, and effectively recognized all the 23 linear peptides assessed. Thus, this specific antibody preparation is more valuable than CCPP to face COVID-19, avoiding the multiple CCPP drawbacks for human use.

Keywords: Antibodies, COVID-19, plasma, SARS-CoV-2, Spike protein

RESUMEN

Análisis de la especificidad del plasma de convalecientes de COVID-19 y de anticuerpos purificados contra péptidos lineales de la proteína espiga del SARS-CoV-2. Es continuo el aumento del conocimiento sobre los epítopos de la proteína S, de la espiga del SARS-CoV-2, y sobre los anticuerpos neutralizantes específicos generados durante la infección natural. La estrategia terapéutica que aplica el plasma de pacientes convalecientes de COVID-19 (CCPP), transfiere pasivamente dichos anticuerpos para combatir la infección. Sin embargo, el CCPP tiene problemas de disponibilidad temporal, bioseguridad, variación en la concentración de anticuerpos neutralizantes específicos, inconsistencias entre lotes e inconvenientes de calidad. En este trabajo, se purificó una mezcla de anticuerpos específicos contra la proteína S, de manera segura, en estado puro y concentrados, a partir de cinco CCPP y mediante cromatografía de afinidad (CA) de proteína A-Sefarosa. Se analizó su especificidad contra 23 péptidos lineales, del dominio de unión al receptor (RBD) de la proteína S, diseñados mediante herramientas bioinformáticas. La mezcla de anticuerpos tuvo una concentración de proteínas de 2,43 ± 0,36 mg/mL y una pureza del 96,10 ± 0,89 % (condiciones reductoras) y del 95,6 ± 1,29 % (condiciones no reductoras). Los CCPP reconocieron solo 13 de los 23 péptidos lineales, contra 23 la mezcla de anticuerpos. Esto corroboró la amplia diversidad de la respuesta de células B, y la posible interferencia de componentes del CCPP en la sensibilidad del ensayo tipo ELISA. Como conclusiones, se plantea que la purificación mediante CA puede no afectar la cantidad de anticuerpos tipo IgG presentes en los CCPP, ni su capacidad para reconocer los 23 péptidos lineales evaluados. Esta preparación de anticuerpos es más valiosa y adecuada que los CCPP, para su uso en humanos.

Palabras clave: Anticuerpos, COVID-19, plasma, proteína espiga, SARS-CoV-2

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*I*ntroduction

Within the past two decades, three highly pathogenic coronaviruses emerged into the human population that can cause severe acute respiratory syndrome (SARS). The severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2002, the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2011 and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 [1].

These coronaviruses have at least four major structural proteins, said the nucleocapsid (N), membrane

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(M), envelope (E) and spike (S). The S protein is integrated over the viral surface and mediates its attachment to the receptor on the host cell membrane, leading to the fusion of the viral and host cell membranes and the release of the viral particle into host cells during entry [2]. Thus, in terms of protection, the S protein is the most relevant viral antigen to induce a specific neutralizing antibody response, so far [3].

On the other hand, blood plasma (blood liquid part) has been used for more than 100 years to fight diseases produced by bacteria and viruses [4, 5]. For instance, plasmas from convalescent patients have been used as an effective tool in COVID-19 patients (CCPP), especially when it is used at the onset of disease [6]. It provides a passive short-term immunity in susceptible people, health care providers, and vulnerable individuals with underlying medical conditions. This strategy was also used to fight SARS-CoV and MERS-CoV outbreaks [7]. Within benefits associated with the CCPP use is mainly those related to antibody-mediated suppression of viremia.

Though, treatment with plasmas has key drawbacks [4]. The most common adverse reactions of CCPP therapy are transfusion-related events, involving chills, fever, anaphylactic reactions, transfusion-related acute lung injury, circulatory overload and hemolysis [8]. In addition, plasmas are associated to batch-to-batch inconsistencies, and the amount of specific and neutralizing antibodies are difficult to quantify. Therefore, the use of highly purified antibody preparations is more valuable than CCPP.

Antibodies have several effector functions that coordinate responses of other immune cells, including T-cells and macrophages to eliminate pathogens [9]. The early use of intravenous antibody preparation, as an adjuvant therapy in patients with COVID-19 pneumonia, was also found to be effective in reducing the use of mechanical ventilation, and promotes the early recovery of patients, thereby reducing the hospitalization period [7].

Human antibodies are Y-shaped proteins, composed of two identical light chains (LC) and two identical heavy chains (HC). In the natural systems, the pairing of one LC with one HC associates with another identical heterodimer to form an intact antibody molecule. The LC and HC of the heterodimer and the two HC of the heterotetramer are linked by disulfide bridges [10]. The rapid crystallization (Fc) fragment of antibodies is recognized by proteins produced by bacteria to block the antibody functionality [11]. Otherwise, this property has been also widely used to purify antibodies using as ligand those proteins produced by some bacteria [12].

In that sense, one of the most effective IgG-type antibody purification methods uses affinity chromatography based on the Staphylococcus aureus Protein A [13, 14]. This protein is a highly stable cell surface receptor, and consists of a single polypeptide chain of 42 kDa, bearing five homologous Ig-binding domains, rich in aspartic and glutamic acids, but devoid of cysteine. It also contains little or no carbohydrate and only 4 tyrosine residues, but not tryptophan. It is capable of binding to Fc fragment of antibodies, especially gamma-immunoglobulins (IgG). One Protein A molecule has been shown to bind at least 2 molecules of IgG simultaneously. The IgG binding domain of Protein A will bind Fc fragment of human IgG subclasses, IgM, IgA and IgE and of mouse IgG1 (weakly), IgG2a and IgG2b. It also binds IgG from other species, including monkeys, rabbits, pigs, guinea pigs, dogs and cats.

Therefore, this work was aimed to analyze the effect of the Protein A-Sepharose antibody purification procedure on the amount of antibodies specific against 23 linear peptides of the SARS-CoV-2 S-protein-receptor binding domain (RBD), as compared to CCPP. These 23 linear peptides were developed by using bioinformatics procedures.

Materials and methods

CCPP isolation

Plasmas were obtained from CCPP, upon anonymous informed consent, and chosen at random from two provinces of Cuba (Havana and Matanzas). Once blood was drawn, it was allowed to clot for 1 h at room temperature. Next, clotted blood was centrifuged for 15 min at 710 × *g* (Hettich Zentrifugen, Kirchlengern, Westfalia, Germany). Subsequently, plasmas were collected, and stored at –20 °C until antibody purification. All CCPP recognized RBD by ELISA.

Purification of CCPP antibodies

Plasmas were diluted (v/v) in 150 mM phosphate buffered saline solution (PBS), pH 8.0 ± 0.2, with conductivity between 11-15 mS/cm. Then, plasma samples were applied to a PD-10 column, loaded with 9 mL of Protein A-Sepharose Fast Flow matrix (GE Healthcare, Uppsala, Sweden), and equilibrated in the same buffer, being operated at 60 cm/h. The column was further washed to the baseline, using the same buffer and linear flow rate. The elution of antibodies was done with 100 mM citric acid, pH 3.0 ± 0.2. The column in the elution step was also operated at 60 cm/h. Next, the elution fraction buffer was exchanged to 20 mM Tris/150 mM NaCl, pH 7.6 ± 0.2, with a conductivity of 11-16 mS/cm in a 16/30 column (GE Healthcare, Uppsala, Sweden), operated at 100 cm/h. Finally, the elution fractions were filtered under sterile condition, through 0.2 µm filters MiniSart (Sartorius Stedim, Sweden), and further analyzed.

Determination of protein concentration

Protein concentration was quantified following procedure described by Lowry *et al*. [15], using bovine serum albumin (BSA) as reference material. The calibration curve was used in the range 100-500 µg/mL.

Estimation of antibody purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sample purity was analyzed by gel electrophoresis on a 12.5 % (w/v) SDS-PAGE followed by a Coomassie brilliant blue R-250 (Bio-Rad) staining, as described by Laemmli [16].

Solid-phase peptide synthesis

Peptide sequences were obtained from the Wuhan SARS-CoV-2 full-length N-protein amino acid sequence (Genbank accession No. MN908947.3), designed using bioinformatic tools and produced at the Synthetic Peptide Unit of the Center for Genetic Engineering and Biotechnology (CIGB) of Havana, Cuba (Table 1). Peptides were synthesized

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on a Fmoc-AM-MBHA resin, by a stepwise solid-phase procedure, using the Fmoc/tBu strategy (Fields GB, Noble RL). Fmoc-amino acids were coupled using DIC/OxymaPure activation and coupling reactions were monitored by the ninhydrin test [17]. The Fmoc groups were removed with 20 % piperidine in DMF. Side-chain protector groups releasing and cleaving from the resin were achieved by the treatment with TFA-H_2O-TIS (95: 2.5: 2.5, v/v) for 2 h. Subsequently, peptides were precipitated with cold ether, dissolved in 40 % acetonitrile/H_2O, and freeze-dried. Crude peptides were purified by reversed-phase-high performance liquid chromatography (RP-HPLC) and the purity, identity and molecular masses were confirmed by Electrospray Ionization Mass Spectrometry (ESI-MS) [18, 19].

Peptide purification by RP-HPLC

The semipreparative purifications of peptides were performed on an AKTA Pure 150 HPLC system (GE Healthcare, USA). Separations were achieved by RP C18 column (Vydac, 30 × 250 mm, 10 µm). A linear gradient from 10 to 52% of solvent B over 60 min and a at 50 mL/min flow rate were used. Detections were accomplished at 226 nm. Solvent A: 0.1 % (v/v) of TFA in water. Solvent B: 0.05 % (v/v) of TFA in acetonitrile.

Analysis of peptide purity by RP-HPLC

Peptides were analytically separated using a RP C18 column (4.6 × 150 mm, 5 µm; Vydac, USA). A linear gradient from 5 to 60 % of solvent B was established over 35 min and at a 0.8 mL/min flow rate. Solvent A: 0.1 % of TFA in water. Solvent B: 0.05 % of TFA in acetonitrile. Chromatograms were obtained at 226 nm, using LabSolutions 5.84 software package for data processing (Shimadzu, Kyoto, Japan).

Enzyme-linked immunosorbent assay for peptide detection

Nunc MaxiSorp plates were coated with 10 µg/mL linear peptides, dissolved in carbonate-bicarbonate buffer, pH 9.6. Then, plates were incubated in a wet chamber at 37 °C for 1 h, washed with 150 mM PBS/0.05 % Tween-20 and blocked with 5% skimmed milk. Subsequently, CCPP and purified antibody samples (1:50 dilution) were respectively applied to the plates, and incubated at 37 °C for 1 h. Then, plates were washed again and incubated under the same conditions with a human anti IgG-Fc conjugated with horseradish peroxidase (Sigma, St. Louis, Missouri, USA). The reaction was developed by adding horseradish peroxidase substrate, and it was stopped with 50 µL of 1.5 M H_2SO_4 per well. Absorbances were measured in a Multiskan ELISA reader (LabSystems Multiskan MS type 352, Finland), using a 450 nm filter.

Statistical analysis

Statgraphics centurion XV.II version 15.2.05 and Microsoft Excel software were used as tools for statistical analysis. Confidence level was set to 0.05.

Results and discussion

CCPP transfer should be of great help to some patients, mainly those who are immunosuppressed, or unable to orchestrate their own antibody responses. For these types of patients, in 2020, the USA FDA granted an approval for plasma use against COVID-19 in humans. Since then, thousands of peoples have been treated with this biological fluid. Some reports have demonstrated its effectiveness at a single dose of 200 mL with a neutralizing antibody titer above 1:640. After transfusion, the level of specific and neutralizing antibodies rapidly increased, and clinical symptoms significantly improved along with the increase of oxyhemoglobin saturation, within 3 days, and absent of severe adverse effects. The therapy was well tolerated and improved the clinical outcomes through neutralizing viremia in severe COVID-19 cases [20].

Though, this application declined, because of mixed results from randomized clinical trials and multiples drawbacks [4]. In this regard, there are several variables that can also affect how well CCPP might work; within them, the determination of the amount of specific and neutralizing antibody, optimal dose and time point, and knowledge about virus variant are imperatives. Thus, the real clinical benefit of this therapy needs further research, in larger and controlled trials. This is also complicate due to the high number of number of people either infected or vaccinated with different variants of SARS-CoV-2.

To overcome these difficulties, here specificity of purified antibodies preparations from CCPP to furtherly supply, instead, a more safety and effective preparation of antibodies to COVID-19 patients. This aspect may be trivial, but it is not clear that all IgG subclasses can be isolated by and recovered from the affinity chromatography matrix.

Results of the antibody response assessed against the 23 linear peptides revealed first that sample of a healthy individual was not able to recognize RBD any of assessed peptides (average absorbance = 0.140 ± 0.031). In consequence the cut-off of the analysis

Table 1. Linear peptides covering 95.5 % of the whole amino acids sequence of the SARS-COV-2 S-protein receptor binding domain (RBD) and the receptor binding motif (RBM)

RBD peptides	
Peptide No.	Sequence
1	PNITNLCPFGEVFNATRFAS
2	NITNLCPFGEVFNATRFASV
3	RFASVYAWNRKRISNCVADY
4	VADYSVLYNSASFSTFKCYG
5	SVLYNSASFSTFKCYGVSPTK
6	STFKCYGVSPTKLNDLCFTN
7	CFTNVYADSFVIRGDEVRQI
8	RGDEVRQIAPGQTGKIADYN
9	DYNYKLPDDFTGCVIAWNSN
10	KLPDDFTGCVIAWNSNNLDS
11	WNSNNLDSKVGGNYNYLYRL
12	YFPLQSYGFQPTNGVGYQPY
13	QSYGFQPTNGVGYQPYRVVV
RBD & RBM peptides	
Peptide No.	Sequence
14	YGFQPTNGVGYQPYRVVVLS
15	NYLYRLFRKSNLKPFERDIS
16	YLYRLFRKSNLKPFERDIST
17	KSNLKPFERDISTEIYQAGS
18	RDISTEIYQAGSTPCNGVEG
19	IYQAGSTPCNGVEGFNCYFP
20	STPCNGVEGFNC
21	CNGVEGFNCYFPLQSYGFQP
22	NGVEGFNCYFPLQSYGFQPT
23	FNCYFPLQSYGFQPTNGVGY

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used to choose a positive response in samples of convalescent patients was (0.234 = 0.140 + 3 × 0.031).

Then, CCPP samples were able to recognize the RBD, and 13 out of the 23 RBD-linear peptides: 1 (0.260 ± 0.031), 2 (0.650 ± 0.261), 3 (0.720 ± 0.532), 5 (0.652 ± 0.280), 6 (0.354 ± 0.117), 9 (0.278 ± 0.20), 10 (0.627 ± 0.737), 17 (0.260 ± 0.031), 19 (0.325 ± 0.35), 20 (0.250 ± 0.060), 21 (0.268 ± 0.035), 13 (0.237 ± 0.024) and 14 (0.260 ± 0.021) (Table 2; Figure 1). This represented 56.5% of the RBD linear peptides and 44.4 % of RBM- linear peptides, corroborating the high diversity of the IgG-type humoral immune response against S-protein RBD epitopes comonly found in COVID-19 convalescent patients. This allowed us to speculated that the lack of recognition of some linear peptides can be produced by the unavailability of specific antibodies, or result from sensitivity issues of the assay using CCPP. In order to corroborate if there were interferences produced by some components of the plasma for peptide recognition, the IgG-type antibodies were subsequently characterized by Protein A-Sepharose CL4B affinity chromatography.

Data for the concentration, amount, yield and purity of IgG-type antibodies purified from five CCPP are shown in table 2. The CCPP volume applied to the affinity chromatography columns ranged 50-100 mL. Meanwhile, the volume obtained after affinity chromatography elution step ranged 65-105 mL, with a 1.51-fold dilution factor, 2.39 ± 0.36 mg/mL average purified antibody concentration. Equivalently, 162-276 mL elution volume and, 2.84-fold dilution factor were obtained for the size-exclusion chromatography. An overall dilution factor equal to 4.14-fold was achieved, with 548 ± 123.4 mg/mL (p = 0.1044) average yields of purified antibodies and 10 ± 3.64 mg/mL CCPP (p = 0.4975).

As expected, average purity of purified antibody preparations as estimated under reducing and non-reducing conditions were 96.1 ± 0.89 % (p = 0.3739) and 95.6 ± 1.29 % (p = 0.3736), respectively (Figure 2). These high values of purity coincided with that of previous reports demonstrating the highly pure antibodies obtained by Protein A-Sepharose chromatography [21]. They also corroborate the inferred interferences against some RBD-linear peptides during CCPP assessment.

This study was done with 23 linear peptides of the RBD, which is located in the Subunit-1of the S-protein (Table 3). The amino acids amount of these 23 peptides (212) covered about 95% of the whole RBD amino acids sequence, including 9 linear peptides of the receptor binding motif (RBM). As it is actually well known, S protein is a multi-domain trimeric glycoprotein composed of two subunits. The Subunit-1 is composed of four domains, including RBD and RBM. On the contrary, the Subunit-2 forms a stalk-like portion of the full-length trimeric protein and is responsible for viral fusion with the host cell membrane [22]. Peptides of RBD were chosen in this study because; antibody responses directed at the RBD have been identified as the main neutralizing component of the SARS-CoV-2 antibody response.

Concerning to this, a reported serological analysis of 536 convalescent healthcare workers revealed that SARS-CoV-2-specific and virus-neutralizing antibody levels are elevated in individuals that experi-

Table 2. Recognition of linear peptides by COVID-19 convalescent patients' plasmas (CCPP) and purified antibody preparations (PAP), with amino acids amounts of peptides (212) covering 95.5 % of the whole amino acids sequence of the SARS-COV-2 S-protein-receptor binding domain RBD and the receptor binding motif (RBM)

	Absorbance at 450 nm										
Molecules	CCPP1	PAP1	CCPP2	PAP2	CCPP3	PAP3	CCPP4	PAP4	CCPP5	PAP5	HI
RBD protein	1.100	1.525	0.701	1.520	0.820	1.560	1.590	1.590	0.390	1.520	0.180
RBD peptides											
1	0.302	0.365	0.283	0.338	0.235	0.423	0.230	0.393	0.250	0.463	0.082
2	0.860	0.455	1.000	1.525	0.478	2.148	0.450	1.233	0.460	1.690	0.181
3	1.665	1.395	0.568	2.748	0.398	3.403	0.464	1.895	0.505	3.100	0.161
4	0.202	0.439	0.188	0.810	0.198	0.940	0.188	0.655	0.185	1.133	0.112
5	0.630	0.479	0.300	1.455	0.955	2.190	0.904	1.203	0.465	1.765	0.176
6	0.403	0.602	0.185	1.700	0.325	2.235	0.325	1.408	0.533	1.863	0.167
7	0.253	0.425	0.195	0.925	0.218	0.933	0.235	0.705	0.210	0.928	0.200
8	0.238	0.460	0.198	0.973	0.208	0.978	0.203	0.830	0.200	0.913	0.142
9	0.293	1.750	0.265	2.053	0.258	1.953	0.305	1.740	0.268	0.145	0.125
10	1.945	2.305	0.354	2.825	0.265	3.063	0.300	2.025	0.273	2.383	0.110
11	0.298	0.967	0.240	1.053	0.218	0.940	0.203	1.100	0.195	1.573	0.115
12	0.143	0.644	0.143	0.865	0.168	0.825	0.160	1.293	0.193	1.300	0.170
13	0.235	1.190	0.213	1.053	0.250	0.963	0.218	0.935	0.270	1.420	0.135
14	0.285	0.404	0.580	0.463	0.248	0.483	0.233	0.520	0.275	0.430	0.128
RBM peptides											
15	0.238	0.860	0.205	1.200	0.240	1.300	0.248	1.153	0.223	1.253	0.113
16	0.230	0.434	0.208	0.828	0.233	0.958	0.215	0.650	0.200	0.870	0.134
17	0.303	0.428	0.283	0.415	0.235	0.503	0.230	0.423	0.250	0.980	0.169
18	0.263	0.952	0.210	0.923	0.218	0.910	0.208	1.030	0.203	2.260	0.126
19	0.518	0.813	0.325	1.665	0.205	2.385	0.193	1.453	0.385	1.828	0.132
20	0.300	0.398	0.288	1.413	0.238	1.615	0.273	1.218	0.150	1.573	0.136
21	0.253	0.368	0.273	0.320	0.260	0.415	0.230	0.383	0.325	0.408	0.128
22	0.253	0.368	0.273	0.320	0.260	0.415	0.230	0.383	0.325	0.408	0.186
23	0.195	0.399	0.183	0.848	0.170	0.895	0.163	0.625	0.180	0.803	0.089

* HI: Healthy individuals.

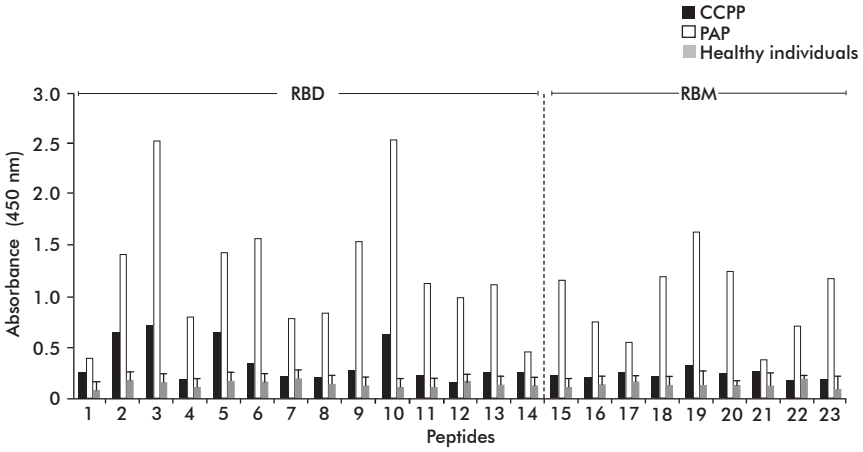

Figure 1. Average absorbance of the linear peptides of SARS-COV-2 S-protein-receptor binding domain (RBD) and the receptor binding motif (RBM) recognized by COVID-19 convalescent patients' plasmas (CCPP), purified antibody preparations (PAP) and healthy individuals.

Table 3. Concentration, amounts, yield and purity of purified antibody preparations (PAP) against peptides of SARS-CoV-2 S-spike-protein*

PAP	CCPP samples	CCPP AC load volume (mL)	Fraction volume (mL)		PAP			SDS-PAGE (%)	
			AC	SEC	Concentration (mg/mL)	Amount (mg)	Ab yield (mg/mL)	R	NR
PAP-1	HC20XHB10-1	100	90	248	1.91	473.8	4.73	95.51	93.50
PAP-2	20MTZ-13	50	65	276	2.41	666.5	13.33	97.00	96.00
PAP-3	20MTZ-11	50	105	236	2.87	677.3	13.54	97.00	96.00
PAP-4	HC20MTZ-19	50	89	162	2.69	435.8	8.70	96.00	95.50
PAP-5	HC20MTZ-7	50	74	239	2.43	486.8	9.73	95.00	97.00
Average	–	–	–	–	2.46	548.0	10.00	96.10	95.60
SD	–	–	–	–	0.36	123.4	3.64	0.890	1.29
P	–	–	–	–	0.7096	0.1044	0.4975	0.3739	0.3736

* Data corresponded to samples collected after size exclusion chromatography (SEC).
CCPP: COVID-19 convalescent patients' plasma. AC: Affinity chromatography. R: Reducing conditions. NR: Non-reducing conditions.

ence severe disease. The severity-associated increase in SARS-CoV-2-specific antibody is dominated by IgG, since Fc-associated functions of IgG are linked with phagocytosis, cytotoxicity and complement deposition, all critical for disease resolution [23]. Therefore, since antibody response of convalescent patients against SARS-CoV-2 proteins is mainly characterized by IgG-type antibodies, purification of IgG from CCPP was performed by Protein A-Sepharose equilibrated in 150 mM PBS, pH 8.0 with a conductivity 11-15 mS/cm and not with 1.5 M glycine/3 M NaCL, pH 8.9 (buffer used to increase binding capacity of Protein A by some low binding classes and subclasses of antibodies). In detail, the Staphylococcal Protein A bind almost exclusively human IgG subclasses (IgG1+++, IgG2+++, IgG3+, IgG4+++), and weakly (+) human IgM, IgA1, IgA2 and Fab. Thus, detection of these classes and subclasses of antibodies were excluded in the study by the use of PBS and the conjugated anti human IgG in the ELISA.

Putting our results into perspective, COVID-19 is a disease characterized by multiple complications, such as a severe lung condition that causes a low amount of oxygen in the bloodstream named acute respiratory distress syndrome, failures in several organs, heart problems, blood clots and acute kidney injury [24]. In COVID-19 treatments, most ill patients can be recovered getting rest, staying hydrated, and taking medications to relieve fever, headache and pain. Nonetheless, treatment choice and its effectiveness will depend on the severity of infection and the comorbidities of patients.

As in the majority of virus diseases, patients may receive antiviral medications; which in general do not kill the virus, but limit production of new viral particles inside host cells shortening duration of illness and lessen medical complications. For instance, Paxlovid and Molnupiravir granted an emergency use authorization by FDA (USA) in December, 2021. Besides, Remdesivir, developed to treat people infected with Ebola and hepatitis C viruses, was also approved by FDA to treat hospitalized adult and pediatric patients with suspected or confirmed COVID-19 [25].

Despite; in severe cases, people require hospitalization. To treat these cases, manufactured monoclonal antibodies (e.g., Sotrovimab, Regdanvimab, REGEN-COV2), administered by intravenous infusion, helped to fight COVID-19 [26]. Furthermore, the emergence of coronavirus mutated variants, caused the Centers for Disease Control in USA to discontinue treatments with some monoclonal antibodies in January 2022.

In 2021, FDA granted an EUA for Tocilizumab to be used as immunomodulator drug in the treatment of adults and children hospitalized with severe COVID-19. This biologic product was formerly approved to treat autoimmune illnesses such as rheumatoid arthritis to reduce inflammation [27]. Jusvinza (synthetic peptide) also received the EUA in 2021 to be used in Cuba in the treatment of severe COVID-19, since it reduces hyperinflammation, hypercoagulation and reestablishes normal values of neutrophils and regulatory T-cells [28]. Besides, the Itolizumab (anti-CD6 humanized antibody) was also authorized in 2021, showing a great effectiveness for the survival of critical and severe COVID-19 patients in Cuba [29]. Application of theses immunomodulatory products in

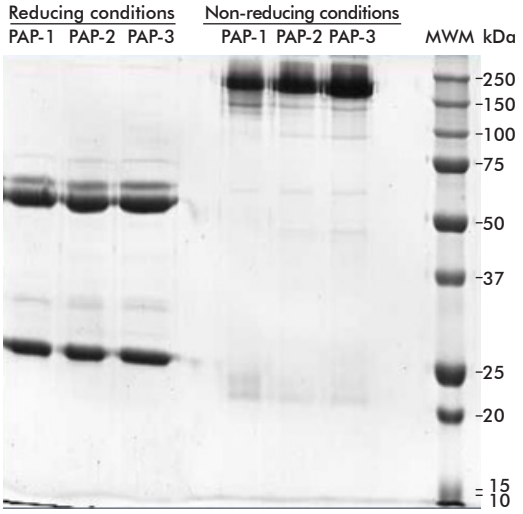

Figure 2. Examples of purity for the purified IgG-type antibody preparations (PAP), as measured by SDS-PAGE under reducing or non-reducing conditions. MWM: Protein molecular weight marker (Catalog number #161-0373; BIO-RAD, USA)

combination with antiviral drugs reduced COVID-19 mortality in patients with the cytokine release syndrome [30].

Dexamethasone is a steroid drug that has been also used to treat inflammation from multiples diseases including asthma, Crohn's disease, cancers and COVID-19 [31]. In COVID-19, it is recommended to be used up to 10 days in hospitalized patients. Nevertheless, more research is needed to confirm the role of dexamethasone as a safe and effective treatment for COVID-19. On the other hand, there are remarkable examples of approved vaccines against COVID-19, which are highly effective at preventing serious disease and death from COVID-19 [32-34]. Yet, the negative issue of this treatment is that these vaccines most be continuously updated to maintain efficacy and effectivity against mutated variants of SARS-CoV-2.

Beyond that, convalescence is a term used to describe the moment of patient recovery from a given diseases (e.g. virus infection diseases). The recovery can be produced by the action of some immunological ccomponents, specialized to fight viruses. Among them, antibodies play a crucial role.

Finally, evaluation of the linear peptide recognition capacity of the purified antibody preparations evidenced a complete recognition of the 23 linear peptides for the purified antibodies (Figure 1). Interestingly, all absorbance values were contradictorily higher than those detected in the CCPP linear peptide recognition capacity assessment. Furthermore, absorbance measured in the ELISA against 13 linear peptides (56.5 %) was higher than 1.0 (2, 3, 5, 6, 9, 10, 11, 13, 15, 18, 19, 20, 23). The largest differences in absorbance values were seen against linear peptides 2 and 10, both out of RBM. Therefore, it is advisable to recommend that the RBD linear peptides assessed in this study should not be tested for humoral response diversity with plasma, neither corroborate the specificity of the antibodies. Probably, some

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plasma components could drastically reduce the ELISA sensitivity. Thus, assessment of the purified antibody preparations highlighted distinct differences in the specific antibody profile against SARS-CoV-2-RBD among assessed convalescent individuals.

Conclusions

Some components of CCPP may affect sensitive of ELISA, which recognized only 13 out of 23 RBD linear peptides, four of them from RBM and nine from RBD. Antibodies purified from CCPP by Protein A Sepharose CL-4B affinity chromatography recognized all of 23 linear peptides of RBD, which means this purification procedure eliminates the assay interference and allows corroborating that IgG type antibodies produced by patients were not IgG3, because this human IgG type is not recognized by Protein A. Therefore, this purified preparation of specific antibodies is more valuable than CCPP to face COVID-19, further avoiding multiples plasmas drawbacks for human use.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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