BIO_11 - Development of Single-Chain Variable Fragment (ScFv) antibody against COVID-19 by phage display as a possible tool to diagnostic and treatment

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Introduction: COVID-19 is an infectious disease caused by SARS-CoV-2, discovered in December 2019. Since then, this highly infectious coronavirus caused a world-wide emergency, with just over 1 year infected more than 100 million of people around the world with almost 2,5 million of deaths Until February 2021, Brazil is third in ranking of infected and second in number of deaths, with more than 9 million of infected and 240 thousand of deaths, respectively. Even with the start of vaccination, the development of therapeutic approaches aimed at reversing severe conditions in patients affected by COVID-19, besides new inputs for diagnosis. Therefore, neutralizing monoclonal antibodies have been emerging as an important alternative to treat cases, with the approval of some of them for emergency use.

Objective: To select fragments of human antibodies from patients affected by SARS-COV-2, using the phage display technique.

Methodology: Twenty-three individuals with confirmed SARS-CoV-2 were recruited for this study (CEP- CAAE 31368620.0.0000.5262). In order to build a human immune library of antibody fragments, peripheral blood mononuclear cells from donors were isolated by Ficoll gradient. From an RNA template, it was produced a pool of cDNA. Heavy and light chains regions were synthesized by PCR to construct scFv genes by overlaping PCR both with specific primers. The scFvlibrary genes were cloned into pCOMB3XSS vector and transformed into *Escherichia coli* XL1-blue. To recover scFv the culture was infected with helper phage VCSM13. To select the target, a recombinant protein was immobilized into 96 wells plate and we have obtained a pool of specific phage-scFvs, confirmed by ELISA. Furthermore, single cells were cultivated and the expression of each scFv was analyzed by dot blot and sequenced by SANGER to identify major prevalence of sequences and the most promising clone was assessed by ELISA against SARS-COV-2 antigens.

Results: The PCR produced 400 bp amplicons for VH and 350 bp for VL and the overlap PCR generated a 800 bp product for scFvs and after four rounds of biopanning was performed and selected scFv against the target. The evaluation of specificity of scFvs was confirmed by high ODs obtained in ELISA tests using phage-scFv and purified scFv. After the evaluation by dot blot of single cells product, the better clone was sequenced and analysed the directly neutralizing activity by PRNT against virus inactivated particle, but was not show effective. However, the PRNT will be repeated using an approach to obtain a complete antibody from this clone.

Conclusion: It was possible to obtain, in record time, an immune library for phage display selection of human fragments. Our panning methodology was successful in selecting specific scFvs fragments against our target, as well as, the whole virus, and could possibly become a tool for diagnosis and treatment for COVID-19

Keywords: SARS-CoV-2; phage display; Antibody