DEVELOPMENT OF A FLOW CYTOMETRY-BASED METHOD TO DETECT NEUTRALISING ANTIBODIES IN SARS-COV-2 INFECTION

Mariana ULINICI

Nicolae Testemitanu State University of Medicine and Pharmacy, the Republic of Moldova

Corresponding author: Mariana Ulinici, e-mail: mariana.ulinici@usmf.md

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Introduction. The ongoing COVID-19 pandemic caused by SARS-CoV-2 has led to significant morbidity and mortality worldwide, underscoring the need for effective diagnostic and therapeutic tools. Neutralizing antibodies are essential components of the immune response to viral infections and play a crucial role in controlling and preventing the spread of SARS-CoV-2. The development of sensitive and specific assays for the detection of neutralizing antibodies is, therefore, crucial for the evaluation of vaccine and therapeutic candidates. In this study, a flow cytometry-based method was developed for the detection of neutralizing antibodies in individuals infected with SARS-CoV-2.

Material and methods. To achieve this, a lentivector system was used to prepare SARS-CoV-2 Spike pseudoviruses expressing Green Fluorescent Protein (GFP) protein. HEK-293T cells were transfected with three plasmids: pLVTHM coding for a GFP reporter, the packaging plasmid psPAX2, and pCDNA3 carrying the D614G SPIKEΔCyto mutation. The infection efficiency was determined by measuring the percentage of GFP-positive cells using a cytofluorimeter (BD Accuri C6) and analyzed using FlowJo software V10 (BD). The neutralization titre was expressed as the reciprocal dilution at which 50% of infection reduction was achieved. The assay was evaluated using 3 cohorts: (i) 100 individuals who recovered from COVID-19; (ii) 100 Sinopharm vaccinated recipients; (iii) 96 pre-pandemic healthy-donors.

Results. After being mixed and pre-incubated for two hours with an equal amount of SARS-CoV-2 pseudotyped lentivirus, samples were examined in duplicate at three different dilutions (1:10, 1:50, and 1:250). To determine the sample's neutralizing antibody content, the mixture was then added to the target cells and incubated for 72 hours**.** According to analyses, the green fluorescence background for untransduced cells ranged from 0.2% to 0.5% across all cohorts. Between 28% and 40% of cells were transduced by the SARS-CoV-2 lentivirus, with or without pre-Covid sera. The outcomes showed high sensitivity and specificity. Moreover, this study demonstrated that the assay could be used to evaluate the neutralizing activity of monoclonal antibodies and convalescent plasma from individuals who have been immunized or recovered from COVID-19. The flow cytometry-based method developed in this study offers a reliable and efficient method for measuring neutralizing antibody responses to SARS-CoV-2 infection. The use of pseudoviruses expressing GFP allows easy detection and quantification of neutralizing antibodies, making it a valuable tool for evaluating vaccine and therapeutic candidates.

Conclusions. Overall, this study provides a significant contribution to the development of effective diagnostic and therapeutic tools against SARS-CoV-2. The flow cytometry-based method offers a reliable and sensitive way to measure neutralizing antibody responses and has potential implications for the evaluation of vaccine efficacy and the development of convalescent plasma therapies. Overall, this method can aid in the fight against COVID-19 and the development of effective interventions to control the spread of the virus.