Khaled Trabelsi

Khaled Trabelsi[1], *Ali Nairouz[1], Nariman Al Mustapha[1], Dana Ashoor[1], Ahmad Ramadan[1], Noureddine Ben Khalaf[1] and Mohamed-Dahmani Fath Allah[1]*

[1] Life Science Department, King Fah Chair for Health Biotechnology, College of Graduate Studies, Arabian Gulf University, Manama 26671, Bahrain

Design, Production and Testing of a Toolbox of SARS-CoV-2 Antigens for Precision Vaccination.

Since the outbreak of SARS-CoV-2 (COVID-19) pandemic in December 2019, the studies aiming on the production of sensitive detection method and producing an effective vaccine were high in the agenda of most of the world researchers. The design of good vaccinating antigens from the SARS-CoV-2 S protein and their production as recombinant good quality proteins at high yield are mandatory steps in the vaccine development process. This is instrumental for the production of a low cost API for the manufacturing of, affordable, effective and safe vaccines. This will likely spur the production of the vaccine doses that would be needed worldwide andparticularly in developing countries, given the likelihood that SARS-CoV -2 becomes endemic We used combined empirical and computational approaches to design a toolbox of recombinants proteins andpolypeptides from the SARS-CoV-2 Spike protein [wild type and mutant versions of the whole protein, the receptor-binding domain (RBD) and others precisely selected subunits) as potential vaccinating antigens. We cloned the corresponding coding sequences in three types of selected vector suitable for the expression in the mammalian cell line HEK293, the yeast Pichia pastoris and E.Coli. We used different experimental design tooptimize the production of each protein in the three-expression systems. We conducted the purification of therecombinants proteins using chromatographic m ethods and the Bradford method to determine protein amounts.We analyzed all the produced protein by SDS-PAGE, silver stain gels and Western blotting. We used different protocols to immunize Balb/c mice with the purified proteins via the intramuscular route. We particularly studied the effect of the injected dose, and the use of different adjuvants such as synthetic oligodeoxynucleotides (CpG), Monophosphoryl lipid A (MPLA), and aluminum hydroxide (Alhydrogel).
The results obtained show that the clones we selected from each of the three-expression system produce good quality, vaccination grade antigens as attested by the high reactivity we observed with SARS-CoV-2 recovering patient's serum. As expected E.coli gave the highest production yield. However, protein produced in HEK293 showed a slightly higher reactivity with patient's sera. Nevertheless, the selection of the best vaccinating antigens is pending upon the analysis of the data from the ongoing immunization experiments for both humoral and cellular immunity. The selected antigen and antigen combination shall be used for the development of a precision vaccineagainst SARS-CoV-2.

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