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Renata G.F. Alvim

Tulio M. Lima

Federico F. Marsili

Leda R. Castilho

TOWARDS UPDATABLE, MULTIVALENT COVID VACCINES: A PLATFORM PROCESS TO PRODUCE TRIMERIC SPIKE PROTEIN OF SARS-COV-2 VARIANTS EXPRESSED IN HEK293 STABLE CELL CLONES

Leda R. Castilho, Cell Culture Engineering Lab. (LECC), COPPE, Federal University of Rio de Janeiro (UFRJ), leda@peq.coppe.ufrj.br
Renata G. F. Alvim, Cell Culture Engineering Lab. (LECC), COPPE, Federal University of Rio de Janeiro (UFRJ)
Tulio M. Lima, EPQB/EQ and Cell Culture Engineering Lab./COPPE, Federal University of Rio de Janeiro
Federico F. Marsili, PPGBq/IQ and Cell Culture Engineering Lab./COPPE, Federal University of Rio de Janeiro

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Within 2.5 years since SARS-COV-2 emergence, COVID-19 pandemic has caused more than 6.3 million registered deaths and 530 million registered cases. The quick development of safe and effective vaccines was very important to mitigate the sanitary crisis. However, the continuous emergence of virus variants with increasing transmissibility continues to cause periodic outbreaks worldwide. The original vaccines keep protecting from severe disease and death caused by variants, but not from transmission and mild disease. Thus, new and improved vaccines are necessary, and updatable, multivalent pan-variant vaccines might be one way to control SARS-COV-2.
The spike (S) protein of SARS-COV-2, a highly glycosylated and very large protein (1380 amino acids), is a key target for diagnostics, therapeutics (e.g. monoclonal antibodies) and vaccines. Very early in the pandemic, Wrapp et al. (https://doi.org/10.1126/science.abb2507) produced the recombinant spike protein as a trimer stabilized in the prefusion conformation and determined its structure by cryoelectron microscopy, providing evidence that it binds to the ACE-2 receptor. The stabilized spike trimer forms the basis of most approved COVID vaccines, across vaccine platforms.
In the present work, we studied the expression and purification of the trimeric prefusion-stabilized spike protein in HEK293 cell lines and developed a platform process applicable to SARS-COV-2 variants (current - and potentially future - ones). Parental HEK293 cells (NRC, Canada or Thermofisher, USA) were transfected by lipofection using Lipofectamine 3000 (Thermofisher Scientific, USA) with a plasmid containing the gene encoding the spike protein. At first, for the ancestral strain (Wuhan), we used a plasmid intended for transient expression kindly provided by VRC/NIAID/NIH (USA). However, for the variants, we ordered synthetic genes (Genscript, USA) that were subcloned in a plasmid intended for stable expression. After genetic modification, stably transfected cells were maintained in the presence of G418 sulfate selection agent. After 3-4 weeks, stable cell pools were obtained and submitted to single cell deposit (FACS Aria, BD Biosciences) in order to obtain clonally-derived cell lines. Documented research cell banks of selected clones were cryopreserved. Batch and fed-batch cultivations were investigated in shake flasks and bioreactors, using the chemically defined HEK TF culture medium and HEK FS feed solution (both Sartorius Xell, Germany). For protein purification, different chromatographic techniques were investigated using Akta Purifier and Akta Pilot systems. Detection of the spike protein secreted in cell culture supernatant was performed by immunoblot, whereas UV280 (Nanodrop, Thermofisher, USA) was used for protein quantitation in purified samples.
After first expressing the spike protein in February 2020 by transient transfection, we developed a stable cell pool by co-transfecting the same transient expression plasmid and an empty stable expression plasmid. This stable cell pool allowed the generation of Wuhan protein that was used to develop serological tests and a hyperimmune equine serum (Cunha et al., doi: 10.1016/j.isci.2021.103315; Alvim et al., in press) and was donated so far to over 90 laboratories in Brazil for basic or applied research.
Subsequently, we developed stable cell lines producing the spike protein of 8 different SARS-COV-2 variants (including the variants of concern beta, gamma, delta, omicron BA.1 and omicron BA.2). For three variants, we also developed clonally derived cell lines, and two more (omicron BA.1 and BA.2) are under way. We keep updating our variant spike panel, and from design and order of a new variant gene to the first miligrams of protein produced by a stable cell pool, we need approximately 60 days. Some agencies (such as Brazilian ANVISA) recommend the use of clonally derived cell lines already for Phase I material, so 5 more months are needed to produce the protein from a clone.
We then developed a platform fed-batch process using chemically defined, animal component-free media, and this fed-batch process was applied to the spike variants expressed so far. Regarding downstream processing, after investigation of different chromatography strategies, we selected a purification process comprising an affinity chromatography capture step that is based on a purification tag, a proteolytic tag cleavage step, and a polishing step combining 3 chromatography techniques *in tandem*. The tag-based process allows the use of the same platform purification process for all tested (and potentially future) spike variants, and enables adequate removal of critical contaminants, such as residual DNA and host-cell protein (HCP). The resulting purified proteins were tested to formulate monovalent and trivalent adjuvanted vaccines. The results obtained so far are highly promising, not only in terms of process development (yield and cost of goods), but also in terms of the preclinical data that our group has obtained so far in immunogenicity, challenge, and repeated-doses toxicology studies