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PROTEIN DESIGN AND IMMUNOGENIC ANALYSIS OF COVID-19 VACCINE CANDIDATES BASED ON RBD/TRIMERIC-SPIKE ANTIGENS AND CHIMERIC VLPS ANTIGENS

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Spike glycoprotein (S) and its Receptor Binding Domain region (RBD) are the main targets of neutralizing antibodies during an infection with SARS-CoV-2, the etiologic agent of COVID-19 pandemic viral disease. Thus, they are the chosen antigens for the development of diagnostic kits and vaccines candidates. Furthermore, Virus-like Particles (VLPs) constitute potent immunogens that have been engineered to obtain vaccine candidates through expression of SARS-CoV-2 S, M, E and N proteins. Although it might be a challenging platform, as multiple proteins must be expressed in order to assure VLP budding, they are able to induce stronger immune responses than soluble antigens due to their particulate nature and highly repetitive antigen display. Hence, we aimed to generate a serum-free platform to produce soluble SARS-CoV-2 antigens and design a novel chimeric VLP (cVLP) exposing the prefusion stabilized S ectodomain on its surface.

First, we generated stables cell lines expressing RBD or trimerized pre-fusion stabilized S as soluble antigens by transfection of HEK293 cells, followed by antibiotic selection. Cell lines were adapted to suspension growth using a chemically defined serum-free medium and cultured in 1 L stirred tank bioreactor operated in perfusion mode for 10 days. Protein expression was assessed along the process by Western Blotting using anti-SARS-CoV-2 monoclonal antibody. Clarified harvests were purified by IMAC reaching greater than 98% purity for both antigens.
Additionally, we designed, expressed and analyzed SARS-CoV-2 cVLPs. To do this, we used rabies glycoprotein transmembrane and cytoplasmic domains as anchor of S ectodomain. Fusion protein expression and its correct plasma membrane localization in HEK293 cells was confirmed by fluorescence microscopy and flow cytometry using anti-SARS-CoV-2 polyclonal and monoclonal antibodies. Moreover, cVLPs were detected on culture supernatants and sucrose cushion ultracentrifugation fractions by western blot, using an anti-RBD monoclonal antibody. cVLP morphology was assessed by negative staining transmission electron microscopy (TEM) and the fusion protein was detected on its surface by immuno-TEM.

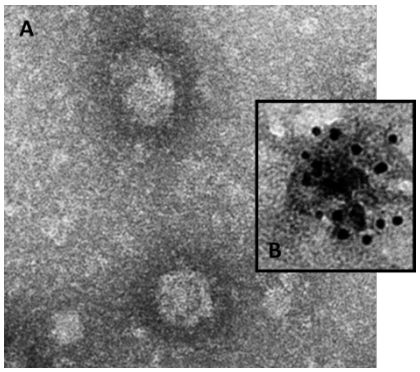

Figure 1. TEM of cVLPs. A. Negative staining. B. Immuno TEM.

Finally, soluble antigens and cVLP immunogenicity was evaluated in mice, following a prime-boost immunization plan with a 21 days gap in between. Balb/c mice were alternatively injected intramuscularly with RBD, S, or the cVLP preparations, containing saponin-based liposomes as adjuvant. RBD and S soluble antigens were formulated with $Al(OH)_3$ or a saponine liposome preparation (LipoSap®), while cVLPs were administered using LipoSap® as adjuvant . Mice were bled 14 days after the boost and sera were analyzed by indirect ELISA. Specific antibodies were detected for the three antigens under evaluation, where cVLPs proved to be highly immunogenic, as they induced similar titres of anti-S antibodies with a significantly smaller amount of antigen per dose.

In conclusion, we were able to obtain immunogenic SARS-CoV-2 soluble antigens through an easily scalable continuous process that can be applied to numerous utilities. Moreover, we designed a novel highly immunogenic cVLP that can be expressed without the necessity of co-expressing other SARS-CoV-2 structural proteins. It presents SARS-CoV-2 native-like morphology and induces humoral response against S in mice, becoming a promising candidate for vaccine applications.