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RATIONAL DESIGN, EXPRESSION AND CHARACTERIZATION OF CHIMERIC RABIES VLPS DISPLAYING THE MAJOR ANTIGENIC SITE OF FOOT AND MOUTH DISEASE VIRUS

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Virus-like Particles (VLPs) are supramolecular arrangements of one or more viral proteins that resemble the structure of the native virus but are not infective, due the fact that they lack the viral genome. They have become very important in the field of novel recombinant vaccines, because of their biosafety and improved immunogenicity over subunit vaccines due to their particulate nature and highly repetitive epitope display. Moreover, they can elicit immune responses against heterologous antigens by the incorporation of epitopes by genic fusion to the viral proteins, constituting chimeric VLPS (cVLPS). In a previous work, by recombinant expression of the rabies G glycoprotein (RVG) in HEK293 cells, we established a platform to produce rabies VLPS as a new generation vaccine candidate 1–3. The main goal of this work was to generate a platform for heterologous antigen presentation based on RVG cVLPS. The heterologous epitope chosen for this study was the immunodominant site of Foot-and-Mouth Disease Virus (FMDV), named G-H loop (that is part of the capsid protein VP1), which is responsible of virus entry into the cell 4.

To define which regions in RVG could be adequate to insert a heterologous epitope, we generated a 3D model of the trimer ectodomain and selected regions without secondary structure, exposed in the surface of the protein, not involved in interactions between the monomers of RVG trimer and with low AA sequence identity between Lyssavirus glycoproteins, indicating some structural flexibility. Three regions were selected as insertion sites, and the heterologous sequence was inserted on each one individually. To obtain stable expression of each of the fusion proteins, suspension growth adapted HEK293 cells were transduced with lentiviral vectors and selected by antibiotic pressure. All of the fusion proteins were correctly localized by flow cytometry and fluorescence microscopy on the plasma membrane, by incubation with an anti-RVG monoclonal antibody and a secondary conjugated to AlexaFluor488. Moreover, the heterologous epitope was exposed in the surface and had an adequate antigenic conformation, as it was detected by anti-FMDV antibodies on each of the fusion proteins. Then, cVLVP budding to culture medium was confirmed by anti-RVG and anti-FMDV ELISA sandwich for each of the variants, and particle morphology was assessed by transmission electron microscopy of ultracentrifugated fractions. Round shape particles with a size of approximately 70 nm were observed through negative staining, and G-H loop was detected on the surface of cVLPS using anti-FMDV antibodies and a secondary conjugated to colloidal gold particles.

Finally, a prime-boost immunization plan of Balb/c mice with each of cVLP variants was performed. Each animal was injected intraperitoneally with the same amount of cVLP, normalized through RVG content, using saponin-based liposomes as adjuvant (LipoSap®). After two doses separated by 21 days, animals were bled, and sera were analyzed by specific indirect ELISA against G-H loop peptide or RVG alternatively. cVLPs were able to trigger antibodies against the heterologous sequence as well as neutralizing antibodies against RVG. Thus, we discovered that rabies VLPS constitute an attracting scaffold for heterologous antigen display which can also protect against rabies virus, potentially becoming a dual vaccine.