Abstract

Thesis title: Recombinant virus-like particles as potential vaccine candidates against Zika virus

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Zika virus (ZIKV) is a human pathogen that belongs to the *Flavivirus* genus in the *Flaviviridae* family. Although most ZIKV infections are asymptomatic, they can sometimes lead to the development of neurological complications, such as Guillain-Barré syndrome. More importantly, the virus can spread through vertical transmission, i.e., from mother to fetus, which may result in the development of congenital zika syndrome (CZS). CZS covers a wide spectrum of birth defects, mainly microcephaly. ZIKV, like other flaviviruses, is mainly transmitted by mosquitoes, which has contributed to its worldwide spread. For many years, ZIKV infections were sporadic, mainly in Africa, until the epidemics in Micronesia and French Polynesia, followed by the 2015-2016 pandemic, which affected almost the entire southern hemisphere. During this period, it is estimated that there were 1,300,000 cases of infection in Brazil alone. Due to climate change, ZIKV continues to spread and is now found in 89 countries around the world. Although the overall number of ZIKV infections is currently low, the virus continues to circulate actively in Latin America and Asia. In addition, recent studies also point to the emergence of new ZIKV strains, that are more infectious and pathogenic.

While many efforts have been made to develop an effective vaccine or antiviral therapy, still none of the above have been approved for human use. Considering the transmission of ZIKV and the risk of another epidemic, as well as neurological complications following ZIKV infection, it remains a serious problem for the human population, especially for pregnant women. Therefore, there is a need for a detailed study of the pathology of ZIKV and, above all, the development of new effective vaccines and antiviral drugs.

Virus-like particles (VLPs) are one type of recombinant antigens used for vaccination purposes. VLPs are structures consisting of one or more different viral proteins, having the ability to self-assemble, thus mimicking native virions. They are devoid of genetic material and are therefore incapable of infecting host cells. Due to that, VLPs have a high immunogenic potential and at the same time are much safer to use as a vaccine component than inactivated virus. For Zika virus, the two viral envelope glycoproteins prM and E are capable of assembling into VLPs, when the genes are expressed together in different eukaryotic cells, e.g., insect or mammalian cells. The process of particle assembly takes place in the endoplasmic reticulum, and then the particles undergo maturation, i.e., glycosylation, proteolytic cleavage and structure reorganization. VLPs produced in different cells may differ in antigenicity, which in turn may influence their immunogenic potential.

This work aimed at examining:

- the influence of the gene expression system and genetic modifications on the process of assembling recombinant VLPs and their antigenicity, as well as determining their immunogenic potential,

- the effect of the dosing regimen and adjuvants on the immunogenicity of selected recombinant VLPs.

In the first stage of the work, genes of different variants of recombinant prM and E proteins were designed, which were then introduced into two gene expression systems: the baculovirus gene expression system in insect cells and the transient expression system in mammalian cells. Gene expression of both proteins was low, therefore the production process of recombinant VLPs was first optimized by modifying the cell culture conditions. In the next step, the ability of prM and E proteins to form VLPs was assessed using the density gradient ultracentrifugation method. In addition, the presence of VLPs was also analyzed by transmission electron microscopy and by dynamic light scattering. In both systems, VLPs were produced at a similar level, but the particles differed in their degree of maturity. As the maturity of the particles, especially glycoprotein E, may affect the immunogenicity of VLPs, the next stage was to analyze the antigenic determinants, i.e., the glycosylation profile of VLPs and lectin binding, as well as the presentation of specific epitopes on the surface of the particles by enzyme immunoassays. The results showed that the prM and E proteins are glycosylated in both systems. However, better binding to lectins of VLPs produced in mammalian cells, as well as better epitope exposure on these VLPs, than on particles derived from the baculovirus system was observed. To compare the immunogenicity of both types of VLPs, two groups of mice were vaccinated with purified VLPs in combination with an oil-in-water adjuvant based on squalene. Immunological analyzes showed a greater ability of VLPs produced in mammalian cells to induce a humoral response and to generate Zika virus neutralizing antibodies. In summary, it was shown that the gene expression system affects the quality of Zika virus-like particles, which translates into their immunogenicity. The mammalian cell system showed superiority in producing mature VLPs with high antigenic and immunogenic potential.

In a second part of the project, the prM/E protein sequences were modified to increase the production of VLPs in mammalian cells. The two-step purification process of the VLPs using chromatographic methods was then optimized. VLPs purified by this method were used for immunological studies to determine the effect of the dosing schedule and adjuvant type on the induction of an immune response in a mouse model. Two vaccination schedules were selected: 3 increasing or decreasing doses of VLPs in combination with the previously used adjuvant administered two weeks apart by subcutaneous administration. In addition, another type of adjuvant was also used - an aluminum hydroxide monophosphoryl lipid A (MPLA) system in combination with VLPs given in an increasing dose schedule. The vaccine formulation following the increasing dose regimen elicited a better IgG antibody response regardless of adjuvant. In addition, VLPs administered with the increasing dose schedule

induced better production of IgG1 and IgG2a subclass antibodies. Sera from mice vaccinated with the increasing dose schedule also showed better neutralizing activity of the Zika virus.

Overall, the results obtained in this study show for the first time differences in the maturity and antigenicity of VLPs produced in different eukaryotic cells. The maturity of the VLPs is of particular importance for use as a vaccine antigen, particularly in eliciting a neutralizing antibody response against Zika virus. Moreover, it has been shown that the immunogenicity of VLPs can be modified by appropriately selecting the dosage of the VLPs and an appropriate adjuvant in the vaccine formulations. This research may contribute to the rational design of vaccine antigens in the form of virus-like particles, their optimal production in eukaryotic cells, as well as the appropriate formulation of the vaccine not only for Zika virus, but also for other viruses from the *Flavivirus* genus.