PURIFICATION OF FLAVIVIRUS VLPs BY A TWO-STEP CHROMATOGRAPHIC PROCESS

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Flaviviruses are enveloped viruses with positive-sense, single-stranded RNA, which are most commonly transmitted by infected mosquitoes. Besides for example dengue viruses (DENV), which have been already for decades posing challenges to public health worldwide, zika virus (ZIKV) and yellow fever virus (YFV) are flaviviruses that have caused significant outbreaks in the last few years. Thus, based on our experience of expressing virus-like particles (VLPs) of several different flaviviruses in recombinant mammalian cells, this work focuses on the development of efficient chromatographic purification processes for zika and yellow-fever VLPs. ZIKV has been discovered in 1947, and since 2007 it has caused isolated outbreaks in Pacific Islands. However, in 2015 it was identified for the first time in Brazil and then quickly spread to over 60 countries between 2015 and 2016. Although most zika patients are asymptomatic, in a small proportion of adults ZIKV infection can cause Guillain-Barré syndrome, and in fetuses of infected women it frequently causes serious congenital malformations, especially in the central nervous system. Since it can be transmitted also by the sexual route and can persist for very long periods in body fluids (including sperm), the development of a vaccine is needed to prevent the spread of the virus to non-endemic countries and to prevent outbreaks to periodically occur in regions where the virus is already circulating.

Yellow fever virus is a highly lethal virus, which causes death in about 6-10% of non-vaccinated individuals. In past centuries, before the introduction of the current live-attenuated vaccine, 10% of the population of cities like Philadelphia (USA) and Barcelona (Spain) died in YF outbreaks. The current vaccine is very safe and provides life-long protection from a single dose. However, it can also cause fatal adverse effects in a small proportion of vaccines, and the egg-based production is limited in capacity. This latter fact led to worldwide vaccine shortages during an outbreak in Africa in 2016 and in Brazil in 2017-2018. Although the WHO introduced during the African outbreak in 2016 the use of a fractional (1/5) dose as an emergency measure to control outbreaks, even if using fractional doses of the current vaccine, shortage would be an issue if YF outbreaks spread and especially if it gets to be locally transmitted in Asia, where the mosquito vector is widespread.

In this work, a two-step chromatographic process was developed for the purification of zika and yellow fever VLPs from CHO- and HEK293-derived cell culture supernatant, building on previous experience acquired on the purification of yellow fever whole virus from Vero cell culture (Pato et al., 2014, doi: 10.1016/j.vaccine.2014.02.036). The initial clarification of the cell culture suspension was performed by centrifugation and/or filtration, followed by anion exchange chromatography and then a multimodal chromatographic step. The anion exchanger used was a Q membrane adsorber, due to its easy scalability, simplicity to handle, absence of diffusional limitations, and good performance at high flow rates for the capture of large molecules such as VLPs. This capture step allowed a high degree of concentration and an efficient DNA removal. In order to enhance HCP removal, a CaptoCore 700 multimodal column was used in a flow-through mode, allowing contaminants to be adsorbed while VLPs were excluded by size. Samples from all steps of the process were characterized by immunoassays, total protein determination, SDS-PAGE and Western blot.
The promising results obtained for zika and yellow fever VLPs indicate that this process could be potentially applied also to other flavivirus VLPs that we have been expressing in our lab, such as DENV1-4, SLEV, CPCV and ILHV. Overall, the presented downstream process could potentially represent a simple, robust and economic platform technology for the production of cell culture-derived recombinant flavivirus vaccines.

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