VAC.07 - Production and characterization of Chikungunya virus for strategic antigen development to support public health demands

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Introduction: The increased prevalence of vector-borne diseases (VBDs), such as Chikungunya fever, has a huge impact on public health worldwide. Vaccines, drugs, diagnostic tests and vector control methods are being developed to prevent, treat and detect VBDs. However, development of such strategies requires a highly knowledge concerning virus infection.

Objective: This work aimed to investigate the kinetic profile of Chikungunya virus (CHIKV) in Vero cells, establish the best virus production conditions in stationary culture and evaluate the growth capacity of CHIKV in serum free media for future immunobiological applications.

Methodology: Vero cells infection by CHIKV was conducted with a Multiplicity of Infection (MOI) of 1.0. Monitoring virus entry, cells were fixed, embedded in polymers, ultrathin cut and analyzed by transmission electron microscopy (TEM). Quantification of CHIKV was performed by real-time PCR (qPCR), for the particles inside the cell and in the supernatant, and by plaque assay for supernatant. Investigating the best conditions for CHIKV production, kinetics were conducted in two MOIs (0.01 and 0.005) for 48 h. Production in serum-free media was also tested. Kinetics were monitored by cytopathic effect (CPE), plaque assay and qPCR.

Results: Data from TEM showed few particles inside the cell at 2 h post-infection (pi) and an extensive CPE with clusters of viral particles into cytoplasmic vesicles associated to the membranes of the rough endoplasmic reticulum at 24 h pi. Besides the enveloped viral particles, nucleocapsids were also observed indicating viral replication. This data is supported by qPCR results in cell fraction, since RNA was initially detected at 4 h pi increasing 3.5 log10 in viral load at 24 h pi. In the supernatant fraction, infectious particles and RNA virus were analyzed and a similar replication profile was observed between 6 and 24 h pi. In CHIKV production kinetics, beginning of CPE was observed in 24 h pi for both MOIs. At 48 h pi, a widespread damage in cell monolayer was observed. Titration data obtained from qPCR and plaque assay reveled maximum virus yields between 29 and 46 h. The MOI for work viral bank was 0.01 resulting in a titer of 7.88 $\log_{10}$ PFU/mL. CHIKV was able to replicate in serum-free media, but the yield was lower than productions in media with serum.

Conclusion: Results showed that Vero cells are susceptible to CHIKV infection, which enables a future vaccine development, since this cell lineage is approved for human usage. The small difference between viral RNA and viral infectious particles suggests the presence of small amounts of defective particles and enables qPCR technique to be used as a marker of infection.

Keywords: CHIKV; viral kinetics; electron microscopy