

IMPLEMENTATION OF A STRATEGY TO PRODUCE A BROADLY NEUTRALIZING MONOCLONAL ANTIBODY AGAINST ZIKA AND DENGUE VIRUSES

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Dengue and Zika viruses are Flavivirus transmitted by a mosquito bite that cause dengue and Zika fever, respectively, with symptoms including fever, rash and headache. The association of Zika virus infection of pregnant women with the development of microcefalia in the fetus lead to the declaration of Zika as a Public Health Emergency of International Concern by the WHO in 2016. The rapid development of methods of detection, quantification and purification of the virus, among others, is necessary for studying the virus at the laboratory and eventually develop a vaccine. Using molecular biology and cell culture techniques, we implemented a strategy to produce a broadly neutralizing monoclonal antibody against dengue and Zika viruses at the lab.

Signal peptides were selected for high level expression of antibodies in CHO cells, according to previous reports (Haryadi et al. 2015). The sequence reported for the human anti-dengue/Zika IgG1 EDE1 C8, including the sequences of the optimized signal peptides for each chain, were cloned into the Freedom™ pCHO 1.0 vector and CHO-S cells were transfected with the resulting construction. Transfection efficiency was low (13%), however, transfected cells were submitted to selection using methotrexate (MTX) and puromycin (PUR) as selection reagents. Two pools of transfected cells were selected using two concentrations of MTX and PUR, and after selection, 100% cells showed expression of the protein of interest, as determined by a parallel control EGFP transfection. Concentration of selection reagents had no effect in productivity in 6-days batch cultures; therefore, the pool of transfected cells growing in the lowest concentration of MTX and PUR was assessed for stability and productivity in presence and absence of selection reagents. Cells growing in medium with and without MTX and PUR showed stable production of the antibody in 10-days cultures, however, differences were found in μ_{max} , X_{max} , and productivity, with the highest values of μ_{max} and X_{max} for the cultures without selection reagents ($\mu_{max} = 0.04 \text{ h}^{-1}$, $X_{max} = 2.68 \times 10^7 \text{ cells/mL}$), in comparison with cultures with selection reagents ($\mu_{max} = 0.03 \text{ h}^{-1}$, $X_{max} = 2.19 \times 10^7 \text{ cells/mL}$). Productivity was higher for cells growing in medium with MTX and PUR ($0.159 \times 10^{-6} \text{ ug/cell h}$) than for cells without selection pressure (0.104 pg/cell h), corroborating the importance of maintaining the selection pressure for optimal expression of the protein of interest in this system. The purified antibody recognized the Zika virus and three serotypes of dengue virus, as observed by dot blot, and according to previous reports that demonstrate that the EDE1 C8 antibody recognizes a quaternary epitope conserved in both viruses (Barba-Spaeth et al. 2016). A more exhaustive evaluation of cell pools is necessary to determine the stability of the expression of the antibody for longer periods of time and to optimize its productivity. This antibody will be used for future research and methods development in our lab. Also, the methodology described here could be used as a start point in the production of other therapeutic antibodies and vaccines.

References:

Barba-Spaeth G, Dejnirattisai W, Rouvinski A, Vaney MC, Medits I, Sharma A, Simon-Loriere E, Sakuntabhai A, Cao-Lormeau VM, Haouz A, England P, Stiasny K, Mongkolsapaya, J, Heinz FX, Screaton, GR, Rey FA (2016) Structural basis of potent Zika-dengue virus antibody cross-neutralization. *Nature* 536: 48-53.
Haryadi R, Ho S, Kok YJ, Pu HX, Zheng L, Pereira NA, Li B, Bi X, Goh LT, Yang Y, Song Z (2015) Optimization of heavy chain and light chain signal peptides for high level expression of therapeutic antibodies in CHO cells. *PLoS ONE* 10: e0116878.

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