

Expression of SARS-CoV-2 Spike Proteins Using Drosophila S2 Cells

Hinano Tsuchiya

Albert To, PhD, John A. Burns School of Medicine Coordinating Center: University of Hawaii at Manoa

ABSTRACT

Declared a pandemic in March 2020, COVID-19 is a disease caused by the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA virus distantly related to SARS-CoV-1, the virus that caused the 2002-2004 SARS outbreak. SARS-CoV-2 poses a more serious global health threat as it is highly transmissible, and causes not only mild to severe symptoms but also asymptomatic infections which makes detection difficult. The spike glycoprotein, on the outer surface of the virus, mediates entry into the host cell through binding to human angiotensin-converting enzyme 2 (hACE2) and is the target for neutralizing antibodies which facilitates viral clearance. In this project, we aim to establish a production system for SARS-CoV-2 spike antigens by using *Drosophila melanogaster* S2 insect cells.

Using a synthetic, codon-optimized gene encoding the Wuhan-Human-1 spike, we conducted targeted polymerase chain reaction amplification and site-directed mutagenesis to produce the receptor-binding domain and full-length spike protein of the initial SARS-CoV2 strain and Beta variant. After transfecting genes into *Drosophila* S2 cells, we established stably-expressing cell lines through antibiotic selection and purified the proteins using monoclonal antibody CR3022 and hACE2 affinity. The production of SARS-CoV-2 antigens in *Drosophila* S2 cells followed by affinity chromatography yields highly pure proteins reactive to both COVID-19 convalescent human and non-human primate sera. This indicates that insect cell-expressed antigens can be used as a diagnostic tool or incorporated into a recombinant subunit protein vaccine with a suitable adjuvant.

KEY WORDS: SARS-CoV-2, Recombinant protein vaccine, protein expression

ACKNOWLEDGEMENTS

The STEP-UP HS program is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, Grant Number: R25DK78386-15.