**Title: Characterization of Unique Pathological Features of COVID-Associated Coagulopathy: Studies with AC70 hACE2 Transgenic Mice Highly Permissive to SARS-CoV-2 Infection**

Drelich AK, Rayavara K, Hsu J, Saenkham-Huntsinger P, Judy BM, Tat V, Thomas G. Ksiazek TG, Peng B-H, and Tseng CTK.

Corresponding author: CTK Tseng ([sktseng@utmb.edu](mailto:sktseng@utmb.edu)), Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, USA

**ABSTRACT**

**Background:** COVID-associated coagulopathy seemly plays a key role in post-acute sequelae of SARS-CoV-2 infection. However, the underlying pathophysiological mechanisms are poorly understood, largely due to the lack of suitable animal models that recapitulate key clinical and pathological symptoms.

**Objective:** To characterize the pathological futures of COVID-associated coagulopathy in hACE2 transgenic (Tg) mice

**Methods:** Fully characterized AC70 line of hACE2-Tg mice with values of LD50 and ID50 of 3 and 0.5 TCID50 of SARS-CoV-2 (WA1 strain), respectively, were challenged intranasally with 105 TCID50 of SARS-CoV-2. These lethally challenged mice were subjected to various analyses for assessing a wide spectrum of host responses, especially virology, hematology, serology, coagulation-related markers, histopathology, etc.

**Results:** Lethal challenge of AC70 hACE2 Tg mice caused acute onset of leukopenia, lymphopenia, along with an increased neutrophil-to-lymphocyte ratio. Importantly, infected animals recapitulated key features of COVID-19-associated coagulopathy, including significantly elevated levels of D-dimer, t-PA, PAI-1, and circulating NETs, along with activated platelet/endothelium marker. Immunohistochemical staining with anti-PF4 antibody revealed profound platelet aggregates especially within blocked veins of the lungs. ANXA2 is known to interact with S100A10 to form heterotetrametric complexes, serving as coreceptors for t-PA to regulate membrane fibrinolysis. Thus, our results revealing elevated IgG type anti-ANXA2 antibody production, downregulated de novo ANXA2/S100A10 synthesis, and reduced AnxA2/S100A10 association in infected mice support an important role of this protein in the pathogenesis of acute COVID-19.

**Conclusion:** We showed that acute SARS-CoV-2 infection of AC70 hACE2 Tg mice triggered a hypercoagulable state coexisting with ill-regulated fibrinolysis, accompanied by dysregulation of ANXA2 system, which might serve as druggable targets for development of antithrombotic and/or anti-fibrinolytic agents to attenuate pathogenesis of COVID-19.

**BIOGRAPHY**

Chien-Te Kent Tseng is a professor in the Departments of Microbiology and Immunology, Pathology, and Cell Biology and the Center of Biodefense and Emerging Disease at University of Texas Medical Branch. Dr. Tseng received his undergraduate training in Plant Pathology at National Chung Hsing University in Taiwan, did his M.S. and Ph.D. thesis and dissertation in the field of Immunology at Mississippi State University and University of Arkansas Medical Sciences, respectively, and did his postdoctoral training in HCV research at University of Texas Medical Brach. Over the past 20+ years, Tseng lab has primarily focused on characterization of the pathogenesis and development of effective medical countermeasures (MCMs) against zoonotic human beta-coronaviruses (b-CoVs) and other respiratory RNA viruses. Among other contributions in this fields, his lab is most well-known for their ability to establish and characterize animal models for studies of pathogenesis of SARS-CoV-1, MERS-CoV, and SARS-CoV-2, and evaluation of immunogenicity, efficacy, and safety of MCMs. His work has been recognized as evidenced by multiple grant and contract awards of various sources.