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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Survival Advantage Associated with Heterozygous Factor V Leiden Mutation in Sars-Cov-2-Infected K18-hACE2 Mice

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The pathogenesis of coronavirus disease 2019 (COVID-19) complications in critically ill patients involves virus-mediated cellular damage in infected tissue and secondary organ damage associated with a hyperinflammatory response. The proinflammatory milieu can result in endothelial dysfunction, predisposing patients to thrombosis leading to a life-threatening condition known as COVID-19-associated coagulopathy (CAC) which is driven by cellular and molecular mechanisms that are still unclear. We developed a mouse model based on the K18-hACE2 transgenic mouse recapitulating many of the CAC-associated pathological findings observed in human patients offering a reliable animal model for the study of SARS-CoV-2 pathogenesis. Plasma analysis revealed virus-induced upregulation of endothelial activation markers and alterations of all the measured coagulation factors' activities, resulting first in increased vascular leakage in the lung on day 5 post-infection, followed by the appearance of focal hemorrhagic lesions potentially associated with local thrombotic events on day 7. The infection-associated procoagulant phenotype appeared as early as day 3 post-infection (p.i.). Prothrombin time showed a 1/3 reduction in the normal clotting time in males. The overall procoagulant state was also reflected in the alteration of Factor(F) VIII and FX activity increasing to ~150% compared to uninfected controls. FIX showed the most significant alteration and was ~200% of the normal activity on day 3 p.i. and gradually increased over time, reaching ~350%. We also observed an increase in the activity of FV starting 3 days p.i. at ~210% of the normal level and persisting unchanged up to day 7 p.i. As reported during human infections, FV activity increased without significant changes in protein concentration or FVa levels. Natural anticoagulant and fibrinolytic mechanisms were dysregulated during virus infection resulting in a profound imbalance in thrombus formation and resolution. Infected K18-hACE2 mice showed downregulation of plasma protein C concentration which was not associated with any alteration of liver function, while plasma protein S concentration remained unaltered. Given the procoagulant environment generated after SARS-CoV-2 infection, reducing activated protein C (APC) anticoagulant and cytoprotective activities by blocking APC's active site with the SPC-54 monoclonal antibody worsened disease outcome and brought the mortality rate from 50% to 90%. In this observed procoagulant pathological context, where we observe increased activity of FV and where a deficiency of APC functions resulted in increased mortality, it wouldn't be surprising to observe an even more severe phenotype with the addition of APC resistance. To test this idea, we generated K18-hACE2 mice harboring the homologous human FV Leiden (FV_L) mutation (R504Q). The Leiden mutation in FV eliminates one of several sites in FVa that are substrates for proteolysis by APC, resulting in diminished inactivation of FVa by APC and preventing the formation of the anticoagulant form of FV that serves as a cofactor for protein S-dependent inactivation of FVIIIa by APC. Upon SARS-CoV-2 infection, the presence of the homozygous FV_L mutation in K18-hACE2 mice didn't affect the severity of the disease as measured by body weight loss during the course of the infection, and homozygous mutated mice had the same mortality rate as FV_{WT} mice. Surprisingly, K18-hACE2 male mice carrying a heterozygous FV_L mutation were extremely protected from SARS-CoV-2 infection, as their mortality dropped from 50% to only 15%. Histological analysis reveals how heterozygous FV_L mice displayed a great reduction in immune infiltrate-associated fibrin deposition, suggesting an unexpected protective role of this prothrombotic mutation in the development of CAC. The protective effect of FV_L heterozygosity may be attributable to enhanced thrombin formation which boosts inflammation and enhanced APC generation controlling proinflammatory and prothrombotic signaling, thus showing a delicate balance achieved by FV_L heterozygosity that was lost for FV_L homozygosity. As seen also in mouse models of bacterial sepsis, such favorable effects associated with FV_L support the somewhat counterintuitive speculation that a partial increase in thrombin production in the early phase of the infection may support the resolution of COVID-19 complications.

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