Role of Fibrinogen and Plasminogen in Neutrophil Infiltration in the Lung in COVID-19

Katheryn N Kapfer[1,2], Kadri Kangro[2], Lisa E Gralinski[3], Nisha Asok Kumar[3], Matthew J Flick[2], Alisa S Wolberg[2]

[1]Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [2]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [3]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

INTRODUCTION

- Severe COVID-19 is caused by SARS-CoV-2 and is characterized by hyperinflammation, multiorgan injury, and thrombotic complications, including stroke.
- Elevated circulating fibrin degradation products and fibrin deposits in the lung and brain have been observed in patients with severe-to-fatal COVID-19.
- Neutrophils have been implicated in COVID-19 pathogenesis through the release of pro-inflammatory cytokines and neutrophil extracellular traps.
- Fibrin(ogen) and plasmin(ogen) can bind to neutrophils and regulate their pro- and/or anti-inflammatory activities, which may influence COVID-19 severity.
- **This study investigates the influence of fibrinogen or plasminogen deficiency on neutrophil infiltration in the lungs and mortality in COVID-19.**

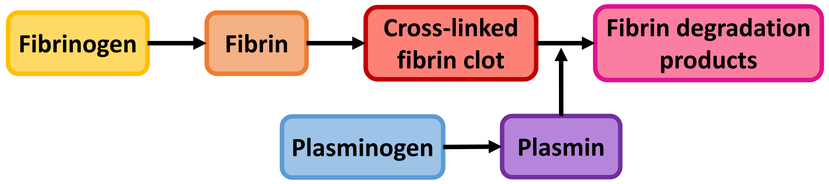

Fig 1. Coagulation cascade highlighting relevant proteins.

METHODS

Male and female 20-week-old C57BL/6J mice sufficient or deficient in fibrinogen (*Fga*$^{+/-}$, *Fga*$^{-/-}$) or plasminogen (*Plg*$^{+/-}$, *Plg*$^{-/-}$) were mock-infected or infected with 10^4 PFU mouse-adapted SARS-CoV-2 (MA10) virus.

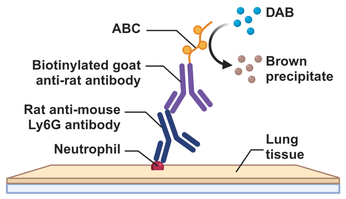

Fig 2. Immunohistochemistry (IHC) scheme. IHC was used to identify Ly6G+ cells (neutrophils) in mouse lung tissue harvested 5 or 30 days post-infection (dpi).

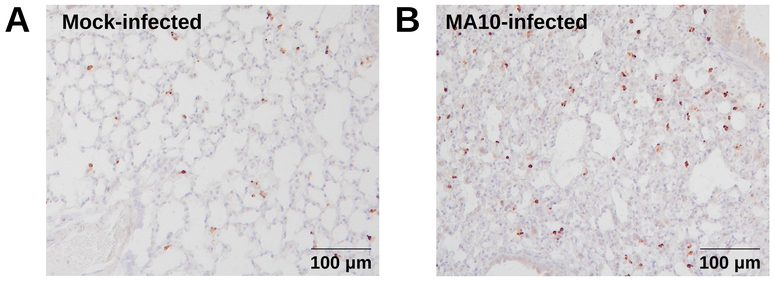

Fig 3. Abnormal alveolar structure and more neutrophils in lung tissue of SARS-CoV-2 MA10-infected mice. IHC staining of neutrophils (brown) on lung tissue sections of 20-week-old male C57BL/6 *Fga*$^{+/-}$ mice mock-infected (**A**) and infected with 10^4 PFU SARS-CoV-2 MA10 (**B**) harvested 5 dpi. Images show 10X magnification.

RESULTS

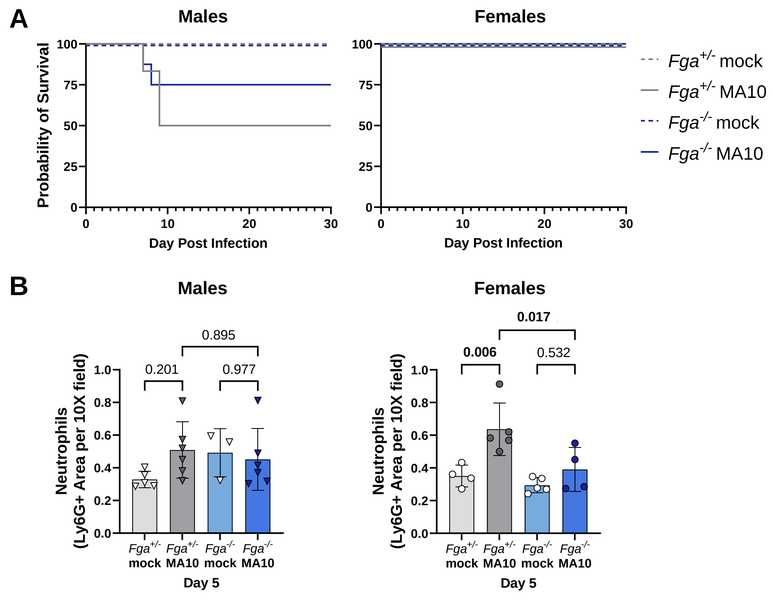

Fig 4. Male and female fibrinogen deficient mice had higher survival, but only females had protection from lung neutrophil infiltration with SARS-CoV-2 MA10 infection. Probability of survival of mock- and MA10-infected male and female *Fga*$^{+/-}$ and *Fga*$^{-/-}$ mice across 30 dpi (**A**)**.** Quantification of average Ly6G-positive area in 10X field of lung tissue sections harvested 5 and 30 dpi from mock- and MA10-infected male and female *Fga*$^{+/-}$ and *Fga*$^{-/-}$ mice (**B**)**.** Survival data analyzed using Kaplan-Meier test followed by log-rank test. Quantification data analyzed by one-way ANOVA and Šídák-corrected. Bars indicate mean ± standard deviation of the mean.

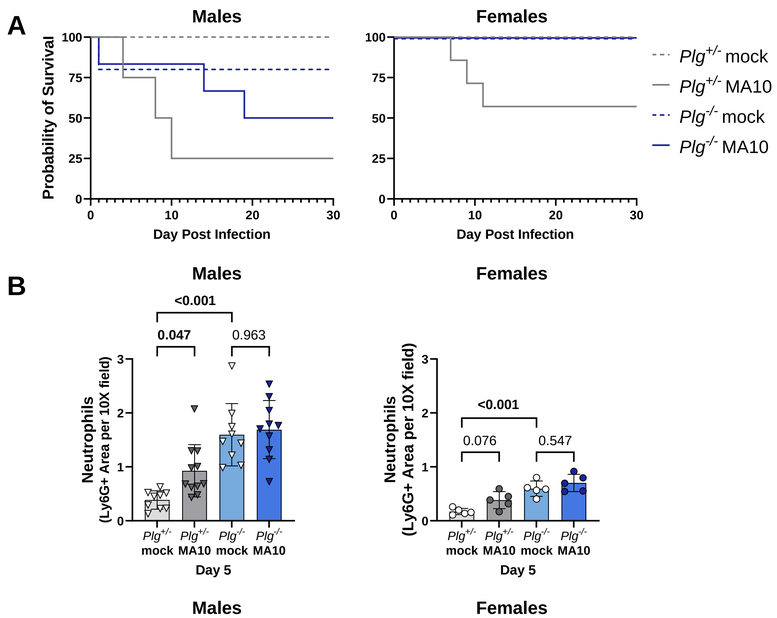

Fig 5. Male and female plasminogen deficient mice had higher survival and more lung neutrophils with SARS-CoV-2 MA10 infection. Probability of survival of mock- and MA10-infected male and female *Plg*$^{+/-}$ and *Plg*$^{-/-}$ mice across 30 dpi (**A**)**.** Quantification of average Ly6G-positive area in 10X field of lung tissue sections harvested 5 and 30 dpi from mock- and MA10-infected male and female *Plg*$^{+/-}$ and *Plg*$^{-/-}$ mice (**B**)**.** Survival data analyzed using Kaplan-Meier test followed by log-rank test. Quantification data from 30 dpi males analyzed by Kruskal-Wallis test and Dunn-corrected. All other quantification data analyzed by one-way ANOVA and Šídák-corrected. Bars indicate mean ± standard deviation of the mean.

CONCLUSIONS

- Overall, female mice had higher survival than male mice, which parallels sex-differences in human COVID-19 survival trends.
- Deficiency in fibrinogen or plasminogen was associated with higher survival with SARS-CoV-2 MA10 infection.
- Lung neutrophil counts were not predictive of survival.
- More studies are needed to elucidate the role of fibrin(ogen)- and plasmin(ogen)-neutrophil interactions in COVID-19 severity and mortality.

ACKNOWLEDGMENTS

2023 Summer Undergraduate Research Fellowship (SURF) Program for Precision Medicine in Health Care (PPMH)

Carolina Virus Research Group

National Institutes of Health

RO1 HL160046

Blood Research Center

CONTACT

kkapfer@unc.edu