

Influence of Fibrinogen and Fibrin on Macrophage and Neutrophil Infiltration in the Lungs in SARS-CoV-2 Infection

Project Aims

Fibrinogen and fibrin (collectively fibrin[ogen]) play a significant role in the immune system hyperactivity and subsequent lung tissue damage seen in severe COVID-19. Fibrin(ogen) interacts with neutrophils and macrophages to promote inflammation, but the extent to which fibrin(ogen) drives the recruitment of neutrophils and macrophages in COVID-19 has not been investigated. I hypothesize that fibrin(ogen) significantly influences macrophage and neutrophil infiltration in the lungs in severe COVID-19 and the extent of this infiltration changes with disease severity. By using immunohistochemistry (IHC) and image processing techniques, I will test this via the following aims:

Aim 1. Compare the quantities of neutrophils and macrophages in the lung tissue of SARS-CoV-2 MA10-infected mice lacking fibrinogen ($Fga^{-/-}$) and mice with sufficient fibrinogen ($Fga^{+/+}$).

Aim 2. Compare the quantities of neutrophils and macrophages in the lung tissue of SARS-CoV-2 MA10-infected mice expressing a truncated fibrinogen α C-region ($Fga^{270/270}$) and mice with sufficient fibrinogen ($Fga^{+/+}$).

Background and Significance

Coronavirus disease 2019 (COVID-19) is a viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 has led to more than 6 million deaths worldwide since its emergence in 2019¹. Death from COVID-19 commonly results from respiratory failure due to acute respiratory distress syndrome, a type of lung injury associated with inflammation and vascular leakage leading to alveolar cell damage and death². In severe COVID-

19, this lung tissue damage is correlated with a cytokine storm, a state of immune system hyperactivity characterized by elevated levels of circulating cytokines that promote a hyperinflammatory response, and coagulation abnormalities associated with fibrin deposition in alveoli³⁻⁵. The protease thrombin converts fibrinogen, a plasma protein, into fibrin, the meshwork that strengthens and stabilizes clots. Fibrin deposition in the lungs and increased levels of circulating fibrinogen and fibrin degradation products have been found in people with severe to fatal COVID-19⁵.

Damage to the alveolar epithelium promotes coagulation and the recruitment of immune cells, including neutrophils and macrophages. Neutrophils fight infection in part, by releasing nuclear material that serves as an extracellular trap that binds resident cells and promotes fibrin mesh formation and deposition within blood vessels, blocking blood flow to tissues^{2,6}. Macrophages release pro-inflammatory cytokines and in severe COVID-19, contribute to the cytokine storm^{7,8}. Both neutrophils and macrophages can interact with fibrin(ogen) through a receptor on their surface (CD11b, Mac-1) and a region of the fibrinogen γ -chain⁹. Engagement of these cells with fibrin(ogen) promotes inflammation. The reciprocal nature of these interactions between fibrin(ogen), neutrophils, and macrophages is thought to contribute to lung tissue damage and respiratory failure seen in severe COVID-19^{2,5}. Preliminary data from our lab suggest neutrophil and macrophage quantities in the lungs of wild-type (WT) mice increase during COVID-19 (Figure 1).

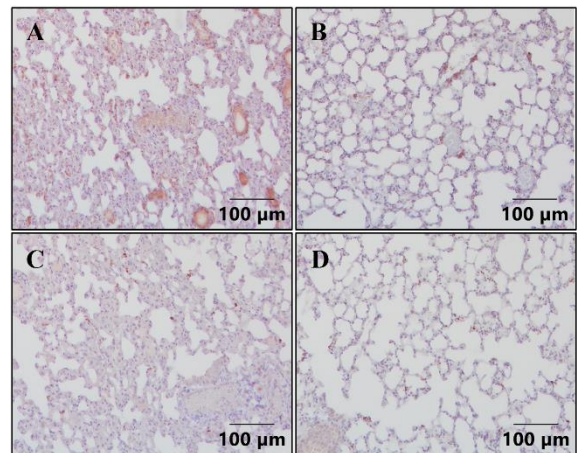


Figure 1. Neutrophil and macrophage quantities increase in SARS-CoV-2-infected lung tissue in mice. IHC of lung tissue of 26-week-old WT C57BL/6J mice infected with the SARS-CoV-2 MA10 variant (A, C) show increased macrophage (A) and neutrophil (C) quantities compared to uninfected mice (B, D). Neutrophils and macrophages are stained in orange. Infected mice also display lung inflammation and irregular alveoli compared to uninfected mice. Tissue was harvested 5 days post-infection.

Since fibrin(ogen) and its interactions with immune cells drive inflammation in many diseases^{2,6,7,9-11}, I hypothesize that interactions of these cells with fibrin(ogen) also drive lung pathology in severe COVID-19. By studying mice lacking fibrinogen or expressing abnormal forms of fibrinogen, I will be able to determine how fibrin(ogen) contributes to neutrophil and macrophage infiltration in the lungs and determine how the quantities of these immune cells are associated with COVID-19 severity. These findings will deepen our understanding of fibrin(ogen)'s role in the hyperimmune response seen in severe COVID-19 and potentially lead to more effective treatments.

Methods

Generation of mouse models. To study the interactions between fibrin(ogen), macrophages, and neutrophils, I will analyze the lungs of male and female $Fga^{-/-}$ and $Fga^{270/270}$ mice infected with the SARS-CoV-2 MA10 variant. I will compare each of these genetic mouse models to littermate fibrinogen-sufficient ($Fga^{+/+}$, $Fga^{+/-}$) mice. These mice have already been bred, infected, and harvested by our lab and our collaborators. $Fga^{-/-}$ have been genetically-engineered to lack the fibrinogen A α chain, resulting in undetectable levels of plasma fibrinogen. $Fga^{-/-}$ mice are unable to produce fibrin, causing spontaneous, yet largely non-life-threatening bleeding events¹². $Fga^{270/270}$ mice have a truncated Fga gene, reducing plasma fibrinogen levels to ~10% to that of WT mice, prolonging clotting times but still allowing for fibrin production and homeostasis maintenance¹³. The SARS-CoV-2 MA10 variant is a mutated strain of SARS-CoV-2 capable of infecting mice and replicating symptoms of mild-to-severe COVID-19 in C57BL/6J and BALB/c mice strains⁴. I will analyze fixed lungs previously harvested from 20-week-old C57BL/6J mice 5 days post infection. I will study 5-6 mice per group (uninfected and infected).

Preparing tissues for IHC. Harvested lungs from each mouse model are preserved in 70% ethanol. The lab manager will place them in an automatic tissue processor overnight to be dehydrated and infiltrated with paraffin wax. The next day, I will place the tissues in a mold filled with melted paraffin wax and leave them to harden on a cooling table. Using a microtome, I will slice the tissues into 5 μm -thick sections and place them on microscope slides.

IHC. IHC involves the use of a primary antibody to target a specific marker for neutrophils and macrophages (Ly6G and IBA-1, respectively) and a biotin-labeled secondary antibody to target the primary antibody, allowing us to visualize the cell marker from the surrounding tissue¹⁴. The sections will undergo standard IHC protocol summarized as follows: 1) deparaffinization and dehydration via heat and submergence in a series of solutions, 2) antigen retrieval via submergence in Rodent Decloaker under pressure, 3) quenching via incubation in hydrogen peroxide, 4) blocking, 5) overnight incubation with primary antibody, 6) incubation with secondary antibody, and 7) secondary antibody labeling through the diaminobenzidine and avidin-biotin complex methods. The sections will then be counterstained with hematoxylin and cover-slipped.

Quantifying neutrophils and macrophages. The tissues will be photographed using a microscope and the images will be analyzed by ImageJ, an image processing program that will quantify the macrophages and neutrophils present in the images.

Timeline. The *Fga*^{270/270} and *Fga*^{+/+} samples have arrived at our lab and the remainder will arrive by April. Tissue embedding and sectioning will be completed by early June, IHC will be completed by late June, and immune cell quantification and data analysis will be completed by early July. The poster and abstract will be completed by mid-August.

Preliminary Work and Experience

I have been a research assistant in [REDACTED] lab since August 2022 and have been working under postdoctoral researcher [REDACTED] [REDACTED]. This past fall, I assisted [REDACTED]

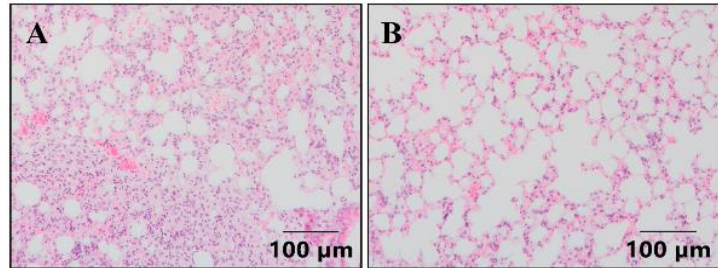


Figure 2. Example of H&E stain. 20-week-old C57BL/6J WT mice lungs were embedded, sectioned, and stained by me. Mice infected with the SARS-CoV-2 MA10 variant (A) show increased lung inflammation and irregular alveoli compared to uninfected mice (B). Tissues were harvested 5 days post infection.

[REDACTED] in her research on fibrinogen in COVID-19 disease and have learned to embed, section, and stain tissues from mice using hematoxylin and eosin (H&E) stain (Figure 2). H&E staining shares many similarities with IHC, but I will practice IHC specifically prior to starting the project in mid-May. I have also become adept at reading scientific literature on COVID-19, fibrinogen, and relevant mice strains, which has increased my knowledge on these topics and improved my ability to analyze our data.

Final Products and Dissemination Plan

I will produce a poster and an abstract illustrating the results of this research and I will submit these to research symposia (e.g., UNC's Pathology Annual Research Symposium) to disseminate my research to a larger audience. The outcomes of this project may be included in a future publication, depending on their significance.

IRB/IACUC Statement

No further approval required.

Total word count: 1194

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