COPD-like Inflammation Induces Neutrophil Invasion and NETosis via the C5a Pathway

Emily Lu[1], Michael J. Clowers[1,2], Atul Sharma[3], Ashwin K. Ramesh[3], Samantha R. Garcia[3], Jyotika Sharma[3], Seyed Javad Moghaddam[1,2]

1 – Department of Pulmonary Medicine, University of Texas MD Anderson Cancer Center (MDACC), Houston, TX. 2 – MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX. 3 – Department of Critical Care, MDACC.

Introduction

- Polymorphonuclear myeloid-derived suppressor cells **(PMN-MDSCs)** are a subset of pathologically activated neutrophils with immunosuppressive activity.
- The pro-tumorigenic activity of PMN-MDSCs may be enhanced by the formation of neutrophil extracellular traps **(NETs)** – weblike structures of DNA-histone complexes extruded by activated neutrophils.
- NET formation, or **NETosis**, has also been implicated in the recruitment of cancer cells to the tumor microenvironment (TME) and in promoting tumor metastasis.
- While stimulatory factors released from the TME – such as neutrophil chemoattractant complement **C5a** – have been characterized as major triggers of NETosis, little is known about the effect of **TME-associated inflammation** in NET formation.

In this preliminary study, we predict that COPD-like inflammation promotes NETosis in the lung tissue via a C5a-dependent mechanism.

Aims

To examine the effect of COPD-like inflammation on NETosis and myeloid cell infiltration in the lung environment.

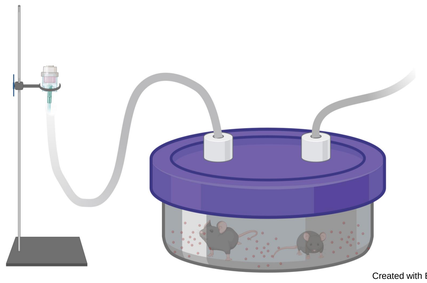
Methodology

Mouse Model

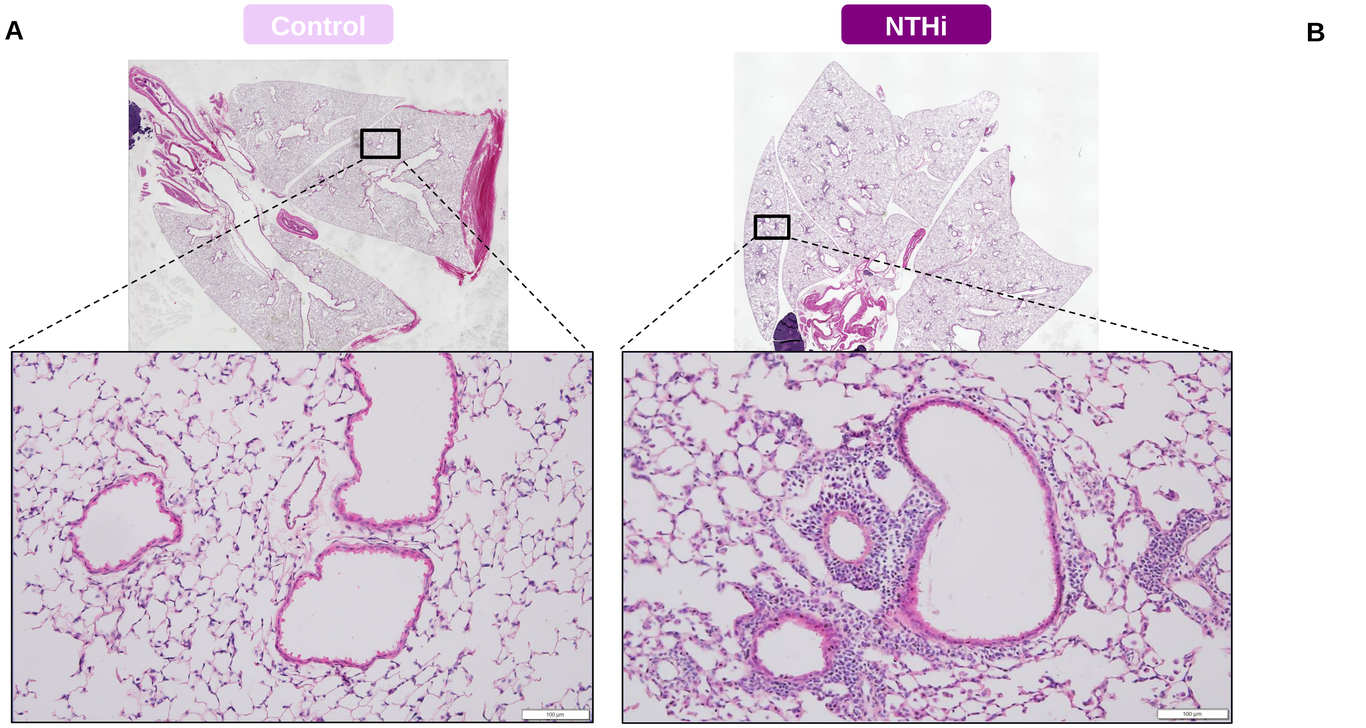
C57BL/6 mice were treated once weekly with 2.5 mg/mL of aerosolized Nontypeable *Haemophilus influenzae* (NTHi) lysate from 6-to-14 weeks of age to induce a COPD-like phenotype.

Methods

Frozen tissue, bronchoalveolar lavage fluid (BALF), and whole lung samples were collected to quantify myeloid cell infiltration, NETosis, and other changes to the lung environment.



Results



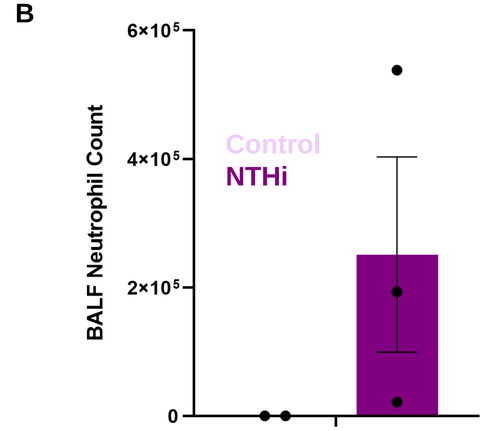


Figure 1. COPD-like inflammation promotes immune cell invasion and neutrophilic influx. (A) Representative compiled photomicrographs of whole lung and zoomed in sections stained with hematoxylin and eosin (H&E); scale bar is 100 µm. **(B)** Quantification of immune cells in bronchoalveolar lavage fluid (BALF) by Wright-Giemsa staining (n=3-5). Data represent mean ±SEM.

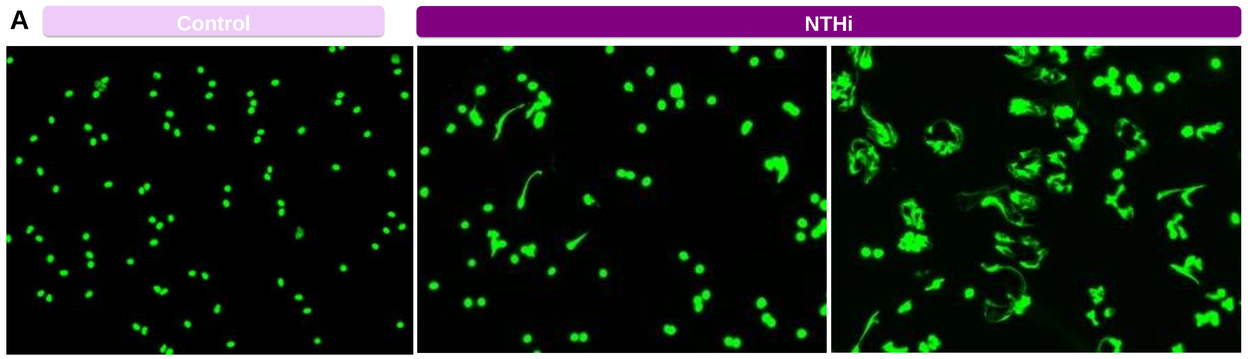


Figure 2. COPD-like inflammation induces NETosis. (A) BALF from wild type (left) and NTHi-exposed (right) mice were stained with Sytox Green Nucleic Acid Stain for NETs (Data were pooled from two experimental replicates). **(B)** Quantification of NETosis, calculated as a ratio of NETs to total neutrophils.

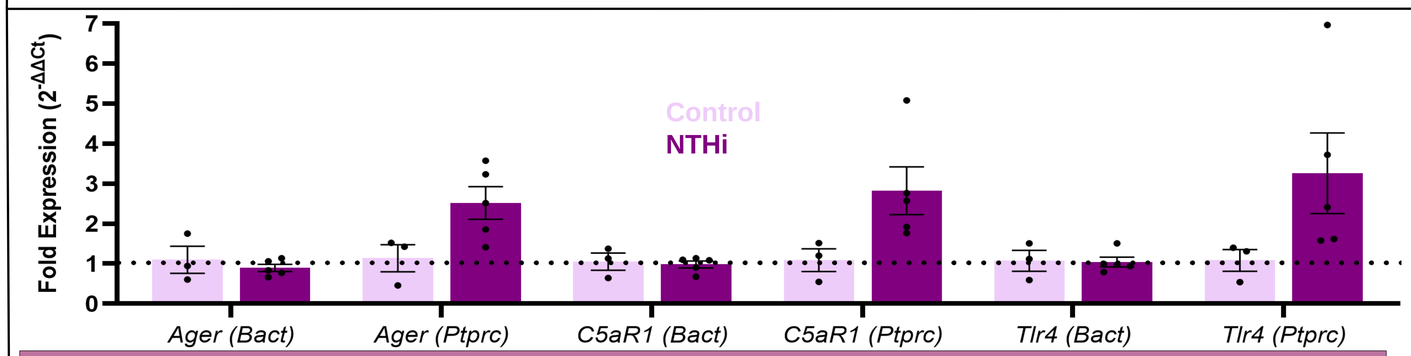
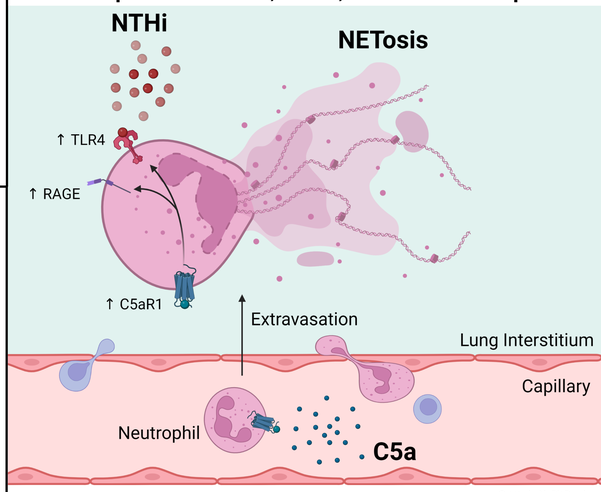


Figure 3. NTHi exposure upregulates transcription of C5a pathway receptors. Quantitative polymerase chain reaction (qPCR) of C5a markers; normalization gene in parentheses; dotted line indicates baseline expression (n=3-5).

Conclusions

NTHi exposure increased neutrophil invasion and NETosis in the airways while also upregulating the transcription of c5aR1, TLR4, and RAGE receptors.



Future Directions

Our next steps include:

- Validating our qPCR results on the protein level
- Expanding the number of treatment groups to examine NTHi exposure and NETosis inhibition with DNase in a lung cancer mouse model (CC-LR).

We hypothesize that NTHi exposure promotes NETosis, tumor cell invasion, and metastases in lung cancer through the upregulation of downstream C5a signaling present on tumor-associated PMN-MDSCs, and that treatment with NETosis inhibitor DNase will significantly reduce these pro-tumorigenic effects.

Confirmation of these results may reveal the potential of NETosis inhibition by DNase as a targeted therapy in the treatment of lung cancer.

Funding

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