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

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### 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) – weiblike 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 (WT) and NTHi-exposed 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.

### 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 positive 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.

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