Investigating the role of platelet factor 4 (PF4) in asthma

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Background

Asthma pathophysiology
- Chronic inflammatory lung disease that affects over 3 million Canadians
- Interleukin-33 (IL-33), interleukin-6 (IL-6), interleukin-8 (IL-8), and thymic stromal lymphopoietin (TSLP) drive inflammation in asthma (fig. 1)

Platelets in asthma
- Platelets play a pro-inflammatory role in multiple diseases
- Platelet factor 4 (PF4) is a pro-inflammatory chemokine released during platelet activation (fig. 2) and is elevated in asthmatic patients

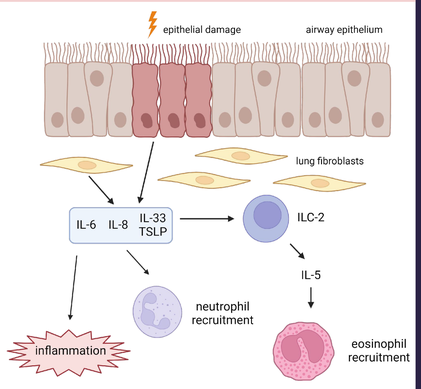

Figure 1. Cytokines that drive inflammation in asthma.

PF4 knockout mice
- In a papain asthma model, PF4 knockout (PF4 KO) mice exhibited less eosinophil recruitment compared to wild type (WT) mice (fig. 3)
- This suggests that PF4 contributes to eosinophil recruitment

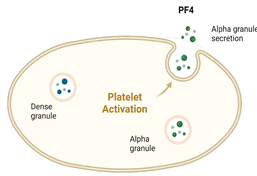

Figure 2. Platelet granule secretion.

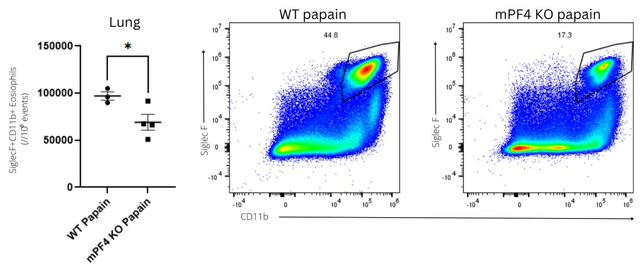

Figure 3 . Eosinophil recruitment in lungs of PF4 KO mice (n = 4) and WT mice (n = 3) measured by flow cytometry after intranasal exposure to papain (t-test, * $p < 0.05$).

Hypothesis & Aims

Hypothesis
PF4 treatment of human lung fibroblasts (HFLs) will increase the cellular production of IL-33, IL-6, IL-8, and TSLP.

Aims
1. To culture HFLs with different concentrations of recombinant PF4 and measure cellular production of IL-33, IL-6, IL-8, and TSLP by enzyme-linked immunosorbent assay (ELISA) and reverse-transcription quantitative polymerase chain reaction (RT-qPCR).
2. To compare immunofluorescence (IF) staining of PF4 and IL-33 between normal and asthmatic lung tissues.

Methods

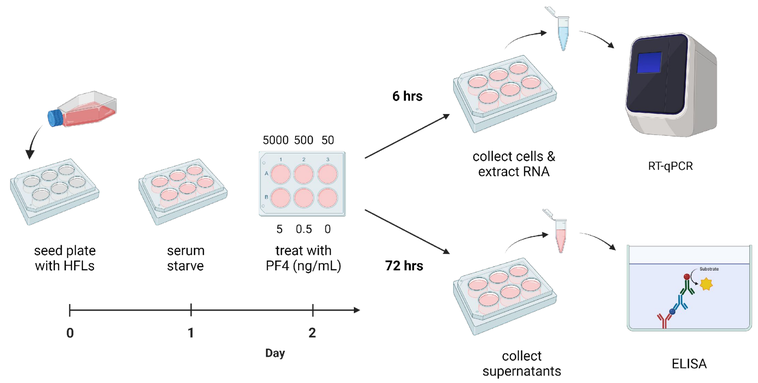

Figure 4. HFL cell culture conditions for RT-qPCR and ELISA experiments.

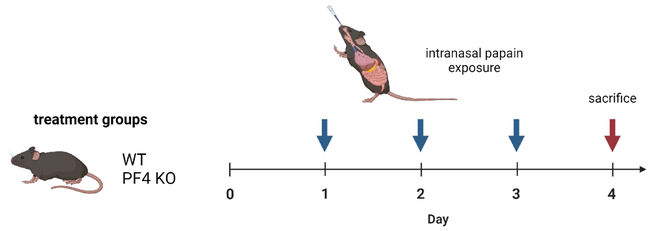

Figure 5. Papain asthma model in WT and PF4 KO mice.

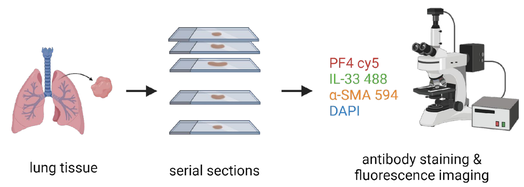

Figure 6. IF staining workflow.

Figures 1, 2, 4-6 made in BioRender

PF4 stimulation drives production of IL-33, IL-6, and IL-8 in HFLs

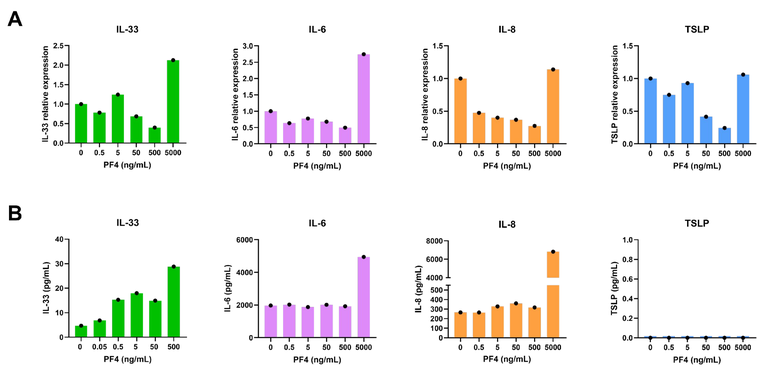

Figure 7. Cellular production of cytokines by HFLs after PF4 stimulation. **A)** mRNA expression and **B)** protein expression of IL-33, IL-6, IL-8, and TSLP (n = 1).

IL-33 staining is increased in lungs of papain-treated WT mice

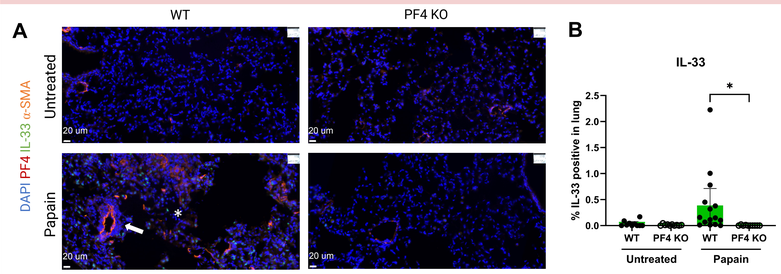

Figure 8. A) Representative IF images of WT and PF4 KO mice frozen lung sections stained for DAPI, PF4, IL-33, and α-SMA. **B)** Quantification of IL-33 staining in WT and KO mice lung sections (one-way ANOVA with Tukey's multiple comparisons, * $p < 0.05$).

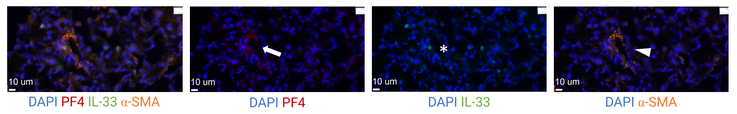

Figure 9. Colocalization of PF4, IL-33, and fibroblasts (α-SMA) in lungs of papain-treated WT mice

PF4 and IL-33 staining is increased in human asthmatic lungs

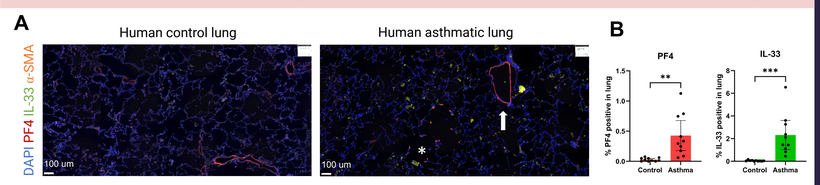

Figure 10. A) Representative IF images of human control and asthmatic FFPE lung sections stained for DAPI, PF4, IL-33, and a-SMA. **B)** Quantification of PF4 and IL-33 staining in human control and asthmatic lung sections (t-test, ** $p < 0.005$, *** $p < 0.0005$).

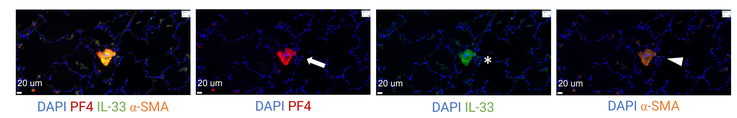

Figure 11. Colocalization of PF4, IL-33, and fibroblasts (α-SMA) in human asthmatic lung.

Conclusions

- mRNA and protein expression of IL-33, IL-6, and IL-8 by HFLs increased in response to PF4 stimulation
- TSLP mRNA expression was unchanged with PF4 stimulation and protein expression was undetectable by ELISA
- IL-33 staining was increased in papain-treated WT mice compared to papain-treated PF4 KO mice, and PF4 and IL-33 staining was increased in human asthmatic lungs compared to control lungs
- Future experiments will investigate the signaling pathways involved in PF4-mediated cytokine secretion

Understanding how PF4 affects cytokine production by lung cells will provide mechanistic insights into the pro-inflammatory role of platelets in asthma

Acknowledgements

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