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

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

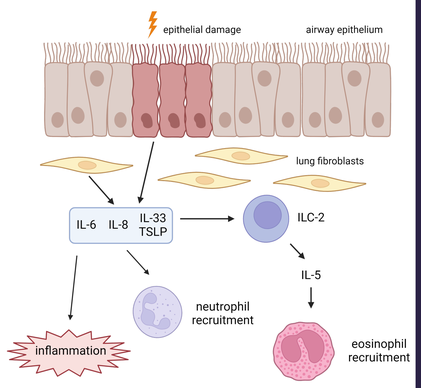

Figure 1. Cytokines that drive inflammation in asthma.

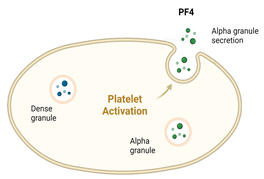

Figure 2. Platelet granule secretion.

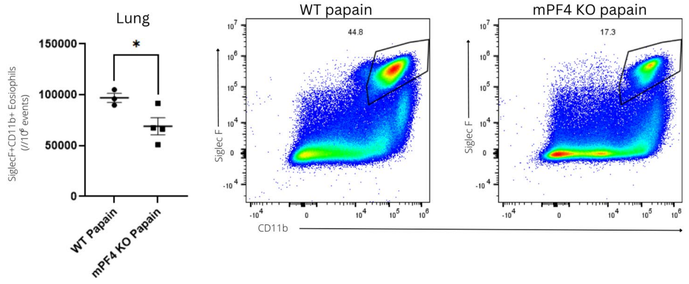

Figure 3 . Eosinophil recruitment in lungs of PF4 KO mice (n = 4) and WT mice (n = 3) measured by flow cytometry after intranasal exposure to papain (* $p < 0.05$, unpaired t-test).

Hypothesis & Aims

Hypothesis

PF4 promotes asthma by increasing expression and secretion of IL-6, IL-8, IL-33, and TSLP by human lung fibroblasts (HFLs).

Aims

1. Compare, via histology, the degree of inflammation and IL-33 staining intensity in the lung tissue of PF4-deficient mice and controls.
2. Compare the spatial localization and staining intensity of PF4 and IL-33 in human lung tissue obtained from asthma patients and controls.
3. Measure the expression and secretion of IL-33, IL-6, IL-8, and TSLP in HFLs cultured in the presence of recombinant PF4.

Methods

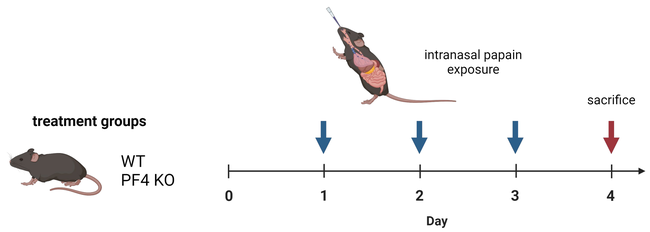

Figure 4. Papain asthma model in WT and PF4 KO mice.

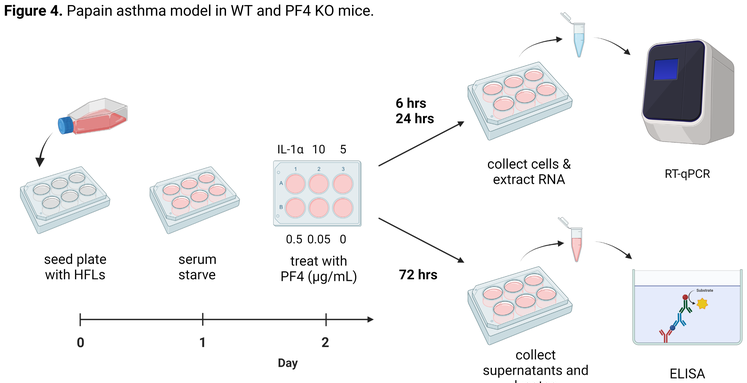

Figure 5. HFL cell culture conditions for RT-qPCR and ELISA experiments.

Figures 1, 2, 4, 5 made in BioRender

Experimental asthma upregulates IL-33 expression in lungs of WT mice

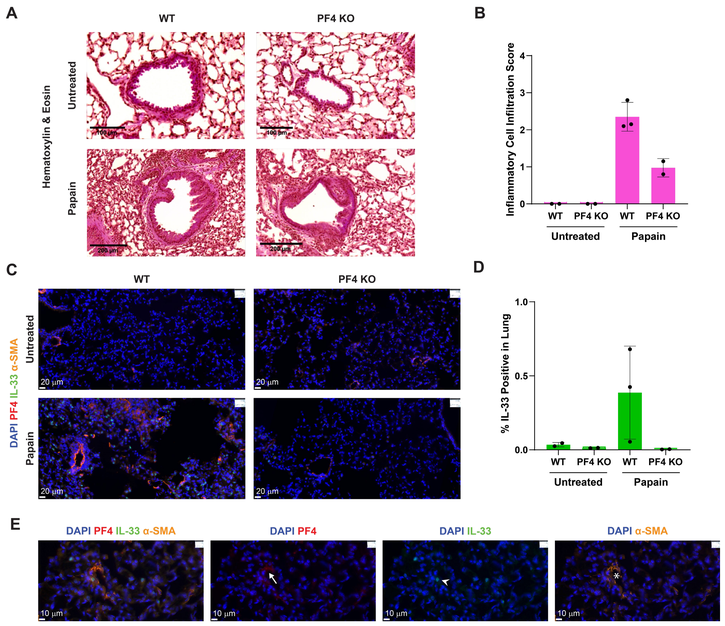

Figure 6. A) Representative H&E images of WT and PF4 KO mice FFPE lung sections. **B)** Quantification of inflammatory cell infiltration in airways of WT and PF4 KO mice. **C)** Representative IF images of WT and PF4 KO mice frozen lung sections stained for DAPI, PF4, IL-33, and α-SMA. **D)** Quantification of IL-33 staining in WT and KO mice lung sections. **E)** Colocalization of PF4, IL-33, and fibroblasts (α-SMA) in lungs of papain-treated WT mice.

IL-33 and PF4 expression is upregulated in human asthmatic lungs

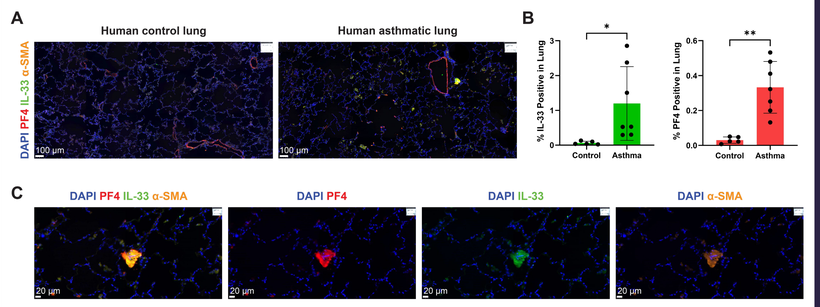

Figure 7. A) Representative IF images of human control and asthmatic FFPE lung sections stained for DAPI, PF4, IL-33, and a-SMA. **B)** Quantification of IL-33 and PF4 staining in human control and asthmatic lung sections (* $p < 0.05$, ** $p < 0.01$, unpaired t-test). **C)** Colocalization of PF4, IL-33, and fibroblasts (α-SMA) in a human asthmatic lung.

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

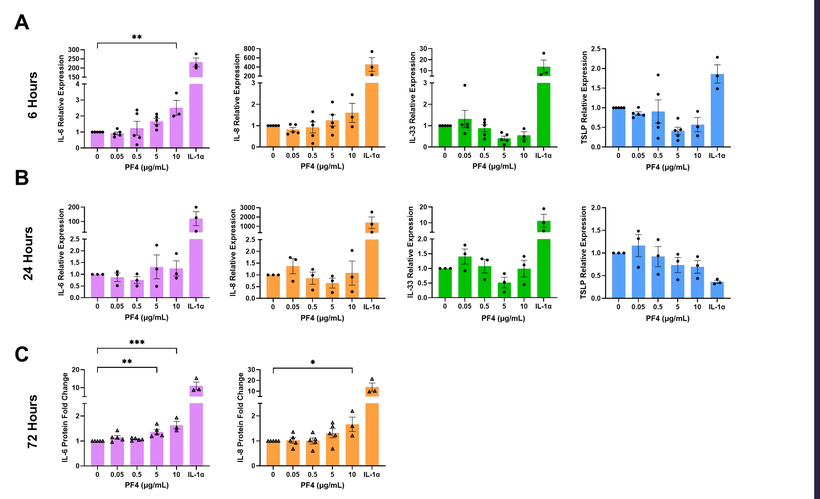

Figure 8. mRNA expression of IL-6, IL-8, IL-33, and TSLP after **A)** 6-hour and **B)** 24-hour stimulation of HFLs with PF4. **C)** Protein secretion of IL-6 and IL-8 after 72-hour PF4 stimulation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, one-way ANOVA with Dunnett's correction).

Conclusions

- Experimental asthma upregulated IL-33 expression in WT but not PF4-null mice
- PF4 and IL-33 expression was upregulated in human asthmatic lungs
- PF4 stimulation of HFLs increased IL-6 and IL-8 expression and secretion
- Future studies on the effects of PF4 on lung epithelial cells may further elucidate the relationship between PF4 and IL-33 in asthma

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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