Phenotypic Characteristics of Eosinophils in COPD

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Introduction

COPD is a disease that affects 65 million people worldwide. A prominent inflammatory disease, COPD is characterised by chronic bronchitis or emphysema, yet the function of eosinophils in the disease remains unknown. Eosinophilic COPD is classified as blood eosinophils consisting of more than 2% of whole blood while non-eosinophilic COPD is characterised as have less than 2% eosinophils in whole blood [1]. Elucidating the phenotypic characteristics of eosinophils in COPD may be important to develop different treatments for the disease as current treatments are too pleiotropic and unspecific.

Hypothesis

Eosinophils exhibit enhanced migration towards IL-5 and eotaxin in COPD patients compared to healthy controls and release more pro- and anti-inflammatory cytokines.

Methods

- Whole blood was harvested from four eosinophilic and four non-eosinophilic COPD patients, and six healthy donors (Table 1).
- Granulocytes were isolated by removing PBMCs by a ficoll gradient and subsequently separating through 3% Dextran.
- RBCs were lysed using a short wash with deionised water. Eosinophils were isolated by negative selection with CD16 MACS beads.
- Eosinophil supernatants were harvested for ELISA or a multi-spot assay after 24hrs.
- Eosinophils were used to measure chemotaxis towards IL-5 and eotaxin.

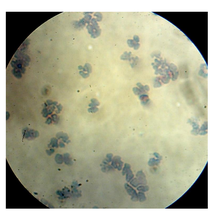

Figure 1: Isolated eosinophils from blood

Results

Table 1: Patient characteristics. *mean (range), #mean (SD)

	Healthy donors	Non-eosinophilic COPD	Eosinophilic COPD
Number of participants	6	4	4
Age (years)*	25 (23-27)	60 (49-76)	61 (53-72)
Gender (M/F)#	2/4	1/3	2/2
Smoker (Current/Ex/Non)#	0/0/6	0/4/0	1/2/1
Blood neutrophils (10^9cells/L)#	3.2 (1.0)	6.2 (1.1)	5.7 (1.2)
Blood neutrophils (%)#	56.3 (5.7)	47.5 (29.0)	64.9 (7.4)
Blood eosinophils (10^9cells/L)#	0.2 (0.1)	0.2 (0.1)	0.4 (0.1)
Blood eosinophils (%)#	2.3 (1.3)	1.7 (1.3)	4.5 (1.5)

Eosinophils from non-eosinophilic and eosinophilic COPD patients exhibited enhanced migration towards both IL-5 and eotaxin compared to healthy donors (mean (SD) healthy donor cell migration to IL-5: 2153 (730.5) compared to non-eosinophilic: 3164 (685.1), p=0.03, and eosinophilic: 7711 (2207), p=0.009. Mean (SD) healthy donor cell migration to eotaxin: 2236 (733.7) compared to non-eosinophilic: 3651 (593.9) p=0.04, eosinophilic: 4952 (745.9) p=0.02 respectively). Eosinophils from eosinophilic COPD patients migrate the most **(Figure 2)**.

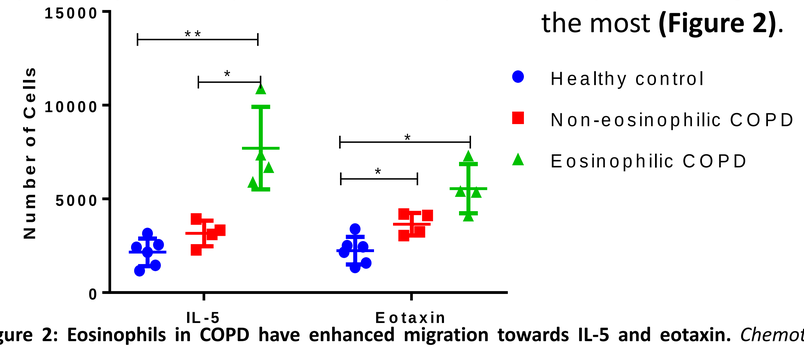

Figure 2: Eosinophils in COPD have enhanced migration towards IL-5 and eotaxin. *Chemotaxis assay of blood eosinophils with IL-5 and eotaxin as stimuli in healthy controls, non-eosinophilic, and eosinophilic COPD patients. Healthy=6, Eosinophilic=4, Non-eosinophilic=4.*

- Greater amounts of pro-inflammatory cytokine IL-1β are produced by eosinophils from eosinophilic COPD patients than non-eosinophilic patients and healthy donors (fold difference to eosinophilic COPD: 6.9 (p=0.04) and 38.9 (p=0.03) respectively), with a trend to increased release of TNF-α (fold difference: 10.9 and 28.6 respectively (p>0.05)), and IL-6 (fold difference: 9.9 and 30.6 respectively (p>0.05)).
- Anti-inflammatory cytokine TGF-β release was higher in eosinophilic COPD than non-eosinophilic COPD and healthy controls (fold difference: 7.3 (p=0.02) and 3.2 (p=0.03) respectively) **(Figure 3)**.

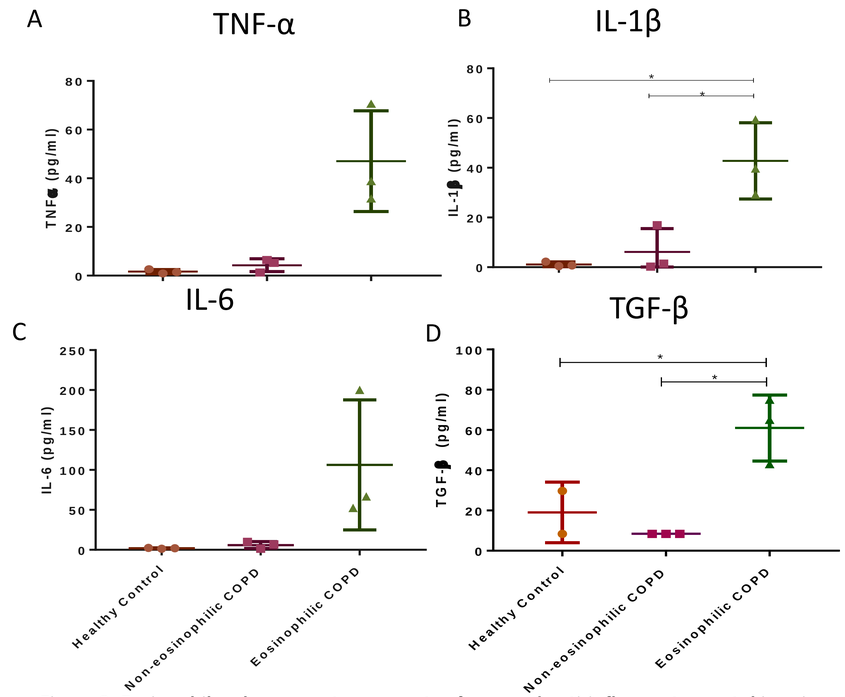

Figure 3: Eosinophils release greater amounts of pro- and anti-inflammatory cytokines in eosinophilic COPD. *Pro-inflammatory cytokines released from blood eosinophils in healthy controls, non-eosinophilic and eosinophilic COPD. (A) TNF-α, (B) IL-1β, (C) IL-6 measured by multi-spot assay. (D) TGF-β measured by ELISA.*

Conclusion

- Eosinophils migrate better under the context of COPD and are capable of secreting larger of pro- and anti-inflammatory cytokines in eosinophilic COPD specifically.
- Eosinophils from COPD patients produce higher levels of pro- and anti-inflammatory cytokines in eosinophilic COPD.
- Future research on the mechanism that induces their phenotype may aid in the development of treatments in eosinophilic COPD.

References

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