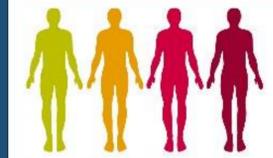




Neutrophil death in a population of patients with neutrophilic COPD

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Introduction

Distinct inflammatory phenotypes of chronic obstructive pulmonary disease (COPD) have been identified; eosinophilic inflammation is a predictor of response to corticosteroid treatment. Patients with non-eosinophilic inflammation do not respond well to corticosteroid treatment, often have raised sputum neutrophils and higher rates of bacterial infections. In normal state, neutrophils undergo apoptosis and are removed from circulation with minimal damage to the system. Necrotic cells release the proteases and reactive oxygen species then contain, amplifying inflammation. Delayed apoptosis in neutrophils from patients with COPD has previously been reported in populations of COPD patients with heterogeneous inflammatory phenotypes. Here we investigate the rate of apoptosis and necrosis in neutrophils from patients with neutrophilic COPD compared to healthy controls.

Hypothesis

We hypothesise that the increased neutrophil counts in those with non-eosinophilic COPD is due to defective apoptosis.

Methods

The necrotic and apoptotic profile of neutrophils from five patients with COPD and five healthy controls were compared. All participants underwent spirometry to assess lung function, and donated blood for the study.

- Blood granulocytes were isolated using Ficoll-paque and 3% dextran solution.
- Granulocytes were plated into 24 well plates at 2×10^6 cells/ml and cultured for up to 40 hours in RPMI, 10% FCS with L-glutamine. In all subjects, granulocytes measured were >95% neutrophils.
- At specified time points, cells were stained with CD16 and Annexin V before measuring with fluorescence activated cell sorting for surface markers of necrosis and apoptosis respectively.
- Neutrophilic, or non-eosinophilic COPD, was defined as eosinophils <2% and neutrophils >60%.
- Data was analysed using 2 way repeated measures ANOVA to compare the amount of apoptosis or necrosis over time. Parametric parameters were compared by unpaired t-test and non-parametric data compared by Mann-Whitney t test.

Results

- Participant demographics are seen in Table 1. One patient with COPD showed eosinophilic inflammation with an eosinophil count of 3% and was excluded from analysis.
- All COPD patients were taking inhaled corticosteroids. The COPD patients had a lower FEV1 compared to controls (mean difference 2.55, 95%CI 1.76 to 3.34, $p < 0.0001$).
- The median (IQR) peripheral blood neutrophil counts in the COPD patients and controls was 10.12 (7.57-11.9) and 3.23 (2.67-3.59) $\times 10^9$ cells/L respectively, $p = 0.0079$.
- No difference was seen in neutrophil death between COPD patients and healthy controls through necrosis or apoptosis at any of the time points tested ($p = 0.349$ and $p = 0.773$ respectively) (Figure 1).

	COPD	Healthy control
Gender (M/F)	1/3	3/2
Age (years)	68 (7.09)	49 (22.88)
FEV1 (L)	1.04 (0.43)	3.50 (0.64)
FEV1% Predicted	49.58 (19.90)	98.58 (16.71)
Smoker? (Current/ex/non)	1/3/0	0/2/3
Blood whole cell count	12.16 (11.10-12.46)	6.09 (4.89-6.47)
Blood neutrophils %	85.99 (65.48-95.11)	53.04 (52.39-57.42)
Blood eosinophils%	1.04 (0.14-1.83)	2.43 (0.88-2.63)

Table 1: Demographics of patients with COPD and healthy controls included in the study.

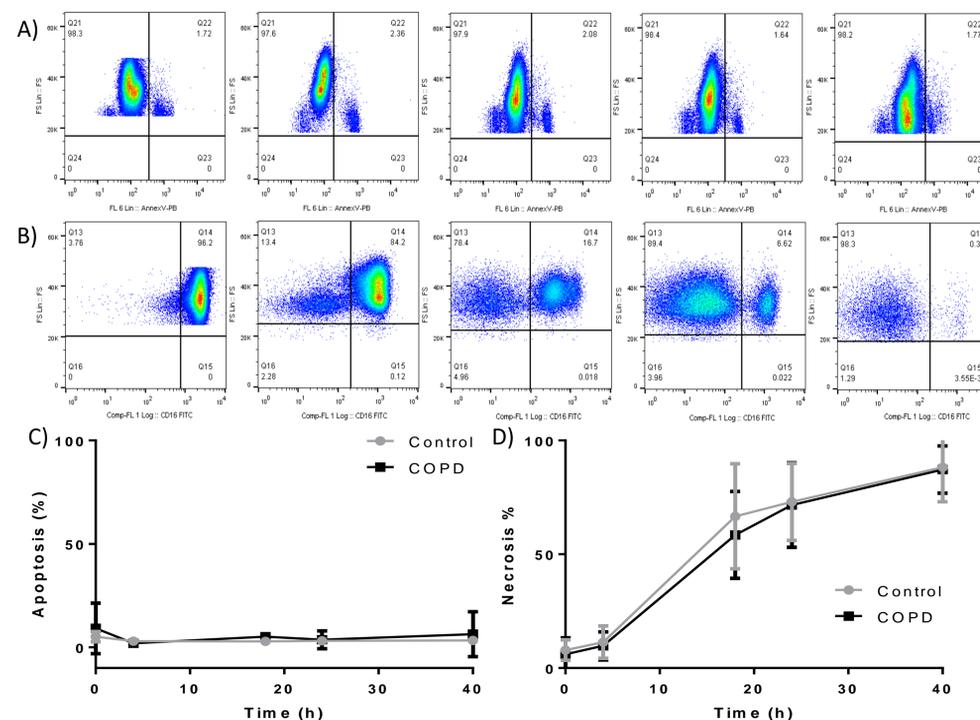


Figure 1: Representative figures of granulocyte A) apoptosis and B) necrosis. Percentage granulocytes C) apoptosing and D) necrosing over 40 hours after isolation from whole blood.

Discussion

- No difference in the rate of necrosis or apoptosis was identified between blood neutrophils from patients with neutrophilic COPD and healthy controls.
- Previous studies have shown a reduction in spontaneous apoptosis rate of neutrophils from patients with COPD, and that this is increased with treatment. As all patients in this study were on steroid treatment, this may be masking such a delay in apoptosis of neutrophils from patients with COPD from being seen.
- A live/dead stain was not included in FACs measures of granulocytes in this study. This means we are unable to distinguish those live apoptosing and necrosing cells from those cells which were dead and therefore no longer carrying out the process but still expressed the apoptotic or necrotic marker.
- Blood neutrophils are clearly not representative of those in the airways, however sputum must undergo processing prior to neutrophil assessment. This decreases the viability of inflammatory cells contained within the sputum therefore measures of apoptosis and necrosis are not representative of the state of the inflammatory cells within the airways.
- While our sample number is small, it is clear from this data that neutrophils from blood of neutrophilic COPD patients and healthy controls follow a similar route of cell death *in vitro*.

Conclusion

The rate at which neutrophils undergo apoptosis or necrosis does not differ between healthy controls and patients with COPD when maintained *in vitro*. This indicates that raised neutrophil levels in patients with COPD do not result from prolonged cell survival. Whether there are differences in airway neutrophil survival needs further investigation.

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