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Title: Detection of STAT1 phosphorylation in COPD sputum by flow cytometry

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Body: Chronic obstructive pulmonary disease (COPD) is an inflammatory disease associated with increased levels of IFNγ which may subsequently contribute to the underlying pathophysiology. IFNγ activates the JAK/STAT pathway via STAT1 phosphorylation leading to increased release of inflammatory mediators; therefore inhibiting this response may provide therapeutic benefit. This pilot study established an intracellular flow cytometry staining protocol to measure STAT1 phosphorylation in sputum cells from COPD patients and healthy smokers. Induced sputum was collected from COPD patients (n=6) and healthy smokers (n=3). Sputum was solubilised with DTT and cells were stimulated with 100ng/ml IFNγ for 20min, fixed with 4% (w/v) paraformaldehyde and permeabilised with ice-cold methanol. A JAK inhibitor (PF956980, 10^{-5}M, 1h) was used to inhibit the JAK/STAT pathway ex-vivo. Flow cytometry was performed and cell populations were determined by their forward scatter/side scatter profile. STAT1 phosphorylation was detected in macrophages from 8/9 samples but not in any neutrophil population. Macrophages from both COPD patients and healthy smokers had similar baseline phosphorylated STAT1 signals (MFI: 250.3±17.9 and 214.4±6.7 respectively). This was increased to a greater extent from COPD patients after IFNγ stimulation compared to those from healthy smokers (MFI 447.9±36.1and 385.4±25.0 respectively). STAT1 phosphorylation in macrophages was completely blocked by PF956980. Sputum is a difficult matrix to perform intracellular flow cytometry staining with and this is the first report of STAT1 phosphorylation being detected in sputum cells from COPD patients and healthy smokers.