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**Title:** Effect of pirfenidone (PFD) on cytokine/chemokine release from alveolar macrophages (AMs) in interstitial lung diseases (ILD): Preliminary results

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**Body:** Rationale: PFD, the first approved treatment for idiopathic pulmonary fibrosis (IPF), exhibits antifibrotic and antiinflammatory activity. The aim of this study is to evaluate the effect of PFD on cytokine/chemokine production by AMs in ILD. Methods: AMs from BAL of 4 ILD patients (2 IPF and 2 nonspecific interstitial pneumonias (NSIP)) were cultured with and without lipopolysaccharide (LPS). The effect of PFD at different concentrations (0.01, 0.03, 0.1, 0.3 mg·ml<sup>-1</sup>) on the production of TNF- $\alpha$ , TGF- $\beta$ 1, IL-1 $\beta$ , IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17, GM-CSF, IFN- $\gamma$ , IP-10, MIP-1 $\alpha$ , MIP-1 $\beta$  and PDGF-BB was tested by Luminex bead based assay (Bioplex, Bio-Rad GmbH, Germany). Results: LPS stimulated the production of TNF- $\alpha$ , IL-1 $\beta$ , IL-8, IL-10, IL-17, GM-CSF, IP-10, MIP-1 $\beta$  and PDGF-BB. PFD suppressed each of these in a dose-dependent manner except for IL-8, IL-17 and PDGF-BB (Table). TGF- $\beta$  was not induced by LPS and pirfenidone did not affect modest basal expression. IFN- $\gamma$ , IL-4, IL-13 and IL-12p70 were not detected in either the presence or absence of LPS. Analysis of the effect of pirfenidone on IL-6 and MIP-1 $\alpha$  is ongoing. Conclusions: PFD seems to reduce the release of TNF- $\alpha$ , IL-1 $\beta$ , IL-10, GM-CSF, MIP-1 $\beta$  and IP-10 from AMs in patients with IPF and NSIP.

Effect of PFD on cytokine/chemokine release from AMs

Cyto/chemokine*		PFD concentration (mg·ml <sup>-1</sup> )				
		0	0.01	0.03	0.1	0.3
TNF- $\alpha$	LPS (+)	404107	174690	96783	41125	18618
IL-1 $\beta$	LPS (+)	1761	1860	1638	1108	666
IL-10	LPS (+)	691	736	685	517	206
GM-CSF	LPS (+)	351	393	327	240	171

MIP-1 $\beta$	LPS (+)	205572	208509	232337	199367	149638
IP-10	LPS (+)	22896	24765	6778	19333	3693

\*Concentration unit: pg·ml<sup>-1</sup>·10<sup>6</sup>Ams<sup>-1</sup>