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Effect of six weeks tobacco smoke exposure on lung macrophage phenotype in mice

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Macrophages have been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Depending on the microenvironment, macrophages can polarise towards either M1 or M2 phenotypes, associated with inflammation or tissue remodelling respectively (Stout et al 2004). Phagocytic capacity is reduced in COPD macrophages (Taylor et al 2010), which we hypothesised may be due to an altered macrophage phenotype as a result of tobacco smoke (TS) exposure.

In this study we investigated the effect of 6 weeks TS exposure on macrophage phenotype in mice and compared this to an acute lipopolysaccharide (LPS) stimulus. Female balb/c mice (n=10 per group) were exposed to TS (450µg/L) for 30min twice a day for 6 weeks. Alternatively mice were dosed intranasally under isoflurane anaesthesia (5%) with 0.3mg/kg of LPS (E.coli 0111:B4) or saline and euthanized 72 and 168 hours later. Animals were terminally anaesthetised (sodium pentobarbital 20mg/kg, i.p) and the trachea cannulated. Bronchoalveolar lavage (BAL) was then performed and a single cell suspension was isolated from the lung tissue by collagenase/DNAse digest (Kirkham et al 2011). . Macrophages, identified as CD11c+/Autofluohigh/F4/80+ were isolated by FACS Aria cell sorter. M1(inducible nitric oxide synthase (iNOS), interleukin-12 (IL-12) and chemokine CXC motif ligand 9 (CXCL9)) and M2 (argininase-1 (ARG1), chemokine CC ligand 2 (CCL2), IL-10 and chitinase 3-like 3 (YM1) marker gene expression was measured using Taqman real-time PCR. Gene expression data was normalised to the house keeping gene ribosomal ribonucleic acid 18S.

Macrophage numbers were elevated in the BAL after 6 weeks TS exposure compared to sham control mice (Cells x 10^3/ml – TS: 54±8 vs. Sham: 35± 3) [Mean ± SEM] $p<0.01$ by a Mann-Whitney test). Similarly an increase in macrophage numbers was observed after LPS exposure (Cells x 10^3/ml – LPS (72 hours): 461±64 vs saline 98±16; $p<0.001$; LPS (168 hours): 323±64 vs saline: 111±16). The change in iNOS and IL-10 expression induced by LPS or TS are shown in table 1. Similar changes were observed with the other M1 and M2 markers measured. Macrophages isolated from the BAL and lung tissue 72 hours post LPS expression both M1 and M2 markers. Compared to saline controls macrophages from the BAL and lung tissue expressed predominantly M2 markers 168 hours post LPS. After 6 weeks TS exposure the macrophages from the BAL express low levels of both M1 and M2 markers. There was an increase in M2 markers in the macrophages from the lungs of TS exposed mice compared to sham controls.

These data suggests that macrophage polarization was altered by TS in a distinctive manner between the BAL and lung tissue. Further studies are necessary to determine whether these macrophages display differences at a functional level. Isolating macrophages from TS exposed animals offers a clear opportunity to investigate molecular and cellular mechanism that may contribute to COPD exacerbation.

Table 1: Effect of TS or LPS exposure on expression of iNOS and IL-10 in macrophages isolated from the BAL and lung tissue of mice

	BAL		Lung tissue	
	iNOS	IL-10	iNOS	IL-10

Sham	0.00±0.00	0.00±0.00	3.32±0.24	144.35±22.33
TS	0.24±0.04	5.93±0.22	6.75±0.40	809.98±2.78
Saline 72 hour	0.03±0.00	0.00±0.00	0.07±0.01	14.59±0.84
LPS 72 hour	17.27±4.09	6.06±1.38	52.60±2.34	556.23±91.66
Saline 168 hour	0.36±0.05	0.00±0.00	0.00±0.00	16.27±1.78
LPS 168 hour	0.29±0.04	0.56±0.06	0.02±0.01	125.57±36.57

Data are expressed at the mean expression normalized to 18S ± standard deviation

Stout et al 2004. J Leukoc Biol 76(3): 509-513; Taylor et al 2010 Eur Respir J 35(5):1039-47; Kirkham et al 2011 AJRCCM 184(7):796-802