Comparison of the pulmonary inflammation induced by a combination of tobacco smoke and lipopolysaccharide or tobacco smoke and haemophilus influenza administration in the mouse

MS Freeman[1], E Partridge[1], D Bayley[1], N Kupfer[1], C Davies[2], EL Hardaker[1], Chris Poll[1]. [1]Novartis, Horsham, RH12 5AB, UK, [2]Cardiff University, School of Pharmacy and Pharmaceutical Sciences, Cardiff, CF10 3NB, UK

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) contribute to increased morbidity and mortality and a decreased quality of life for patients with COPD. Exacerbations, which are often associated with bacterial infection, are defined as a worsening of symptoms that result in a need to alter medication. AECOPD are characterised by a reduction in lung function and quality of life, and are associated with an increase in pro-inflammatory mediators in the lung. Our aims were to develop animal models that would mimic aspects of the altered inflammatory response observed during a bacteria induced exacerbation. Also to compare the use of the bacteria mimetic lipopolysaccharide (LPS) with non typeable haemophilus influenza (NTHi) infection in mice that had been exposed to acute tobacco smoke (TS). Each day for the first two days female Balb/c mice (n=8-10 per group) were exposed twice a day to 30 minutes of either TS (450µg/l wet total particulate matter) or room air (RA). On the morning of day three, mice were intranasally dosed, under isoflurane anaesthesia, with LPS (0.3mg/kg; E.coli 0111:B4) made up in saline (0.9%w/v), NTHi (1 x 109 colony forming units (CFU)/ mouse) formulated in brain heart infusion (BHI) broth or their respective vehicles. Five hours later animals were exposed again to either TS or RA. Twenty four hours after LPS or NTHi administration animals were terminally anaesthetised (sodium pentobarbital 200mg/kg i.p.) and the trachea cannulated. Bronchoalveolar lavage (BAL) was then performed to assess cell influx and cytokine levels. Data are expressed as the mean ±SEM with n=8-10 animals per group. TS exposure does not affect neutrophil infiltration in response to NTHi. Interleukin-1β and interleukin-6 were statistically significantly elevated in TS/NTHi treated animals when compared to RA/NTHi mice (Table 1). Animals exposed to LPS and TS show similar fold changes in cytokines when compared with mice exposed to LPS only, with IL-1β reaching statistical significance ($p<0.01$). Exposing mice to TS prior to NTHi infection appears to cause an exaggerated cytokine response when compared to RA/NTHi treated animals. LPS is well documented to signal predominantly through toll like receptor 4 (Lu et al, 2008). The pattern of pulmonary inflammation was similar in TS/NTHi treated animals to those exposed to TS/LPS, therefore NTHi may elicit many of its pro-inflammatory actions through the same receptor.

Table 1: Effect of NTHI and tobacco smoke on cell numbers & cytokines in the BAL fluid

Treatment groups	**RA/BHI**	**RA/NTHi**	**TS/BHI**	**TS/NTHi**
Neutrophils $x10^3$ml	10±4	1539±182	406±64	1738±316
Interleukin-1β pg/ml	3±5	189±13	43±12	598±174 ###
Interleukin-6 pg/ml	67±14	1982±315	100±21	4473±743 #
Keratinocyte Cytokine pg/ml	2±1	77±5	2±1	194±19

Data expressed as mean ± sem. (Mann Whitney U test comparing TS/NTHi with RA/NTHi, #=$p<0.05$, ## = $p<0.01$ & ### = $p<0.001$.)

Lu Y, Yeh W and Ohashi PS (2008). Cytokine 42 (2): 145-151.