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Title: TLR2 and TLR4 induced tolerance in alveolar macrophages; differential effect on TNFα and IL-8 release

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Body: Alveolar macrophages (AM) play a key role in the pathogenesis of COPD. Bacterial stimulation of toll-like receptors (TLR) on AM causes secretion of inflammatory mediators such as IL-8, promoting neutrophil migration to the lung. Repeat stimulation of TLR results in a reduced inflammatory response, known as tolerance. To emulate events during bacterial colonisation of the lung, we investigated the effects of repeated TLR stimulation in AM from COPD subjects. AM from healthy (n=7) and COPD (n=9) patients were isolated. For tolerance induction, treatment with media, LPS (1µg/ml) or Pam3CSK4 (0.1µg/ml) was performed over a 24 hour period. Cells were then washed before a repeat stimulation. Levels of IL-8 and TNFα were measured post-treatment. Repeat LPS exposure reduced TNFα production but had no effect on IL-8 release from AM (table 1). Differences between healthy and COPD AM responses were negligible. Pre-incubation with LPS before Pam3CSK4 stimulation appeared to prime cells for further IL-8 production compared to levels secreted after two consecutive Pam3CSK stimulations.

Table 1. TNFα and IL-8 Release Following LPS-induced Tolerance in COPD AM

1ST 24 HR	MEDIA	MEDIA	LPS
2ND 24 HR	MEDIA	LPS	LPS
TNFα ng/ml (s.e.m)	0.01 (0.007)	11.2 (2.9)	3.0 (1.8)*
IL-8 ng/ml (s.e.m)	6.6 (2.0)	299.4 (42.7)	352.7 (64.5)

Media+LPS V LPS+LPS *p<0.05

Chronic LPS exposure in COPD lungs results in diminished TNFα production but continual IL-8 release. This may cause neutrophil recruitment and inflammation, further contributing to disease pathogenesis.