

BTS/BLF/BALR Young investigators symposium

T1 REV-ERB α , A NOVEL ANTI-INFLAMMATORY TARGET, MODIFIES THE CIRCADIAN OSCILLATION OF PULMONARY INFLAMMATION

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¹J Blaikley, ¹L Green, ¹J Gibbs, ²S Farrow, ¹A Loudon, ¹D Singh, ¹D Ray. ¹Manchester University, Manchester, UK; ²GlaxoSmithKline, Stevenage, UK

Introduction Most inflammatory diseases demonstrate diurnal variation in severity, for example, chronic obstructive pulmonary disease (COPD) and asthma. We now show that the macrophage inflammatory response is regulated by the circadian cellular clock through Rev-erb α , a nuclear receptor. Additionally, a Rev-erb α ligand (GSK414112) regulates a distinct cytokine network in COPD alveolar macrophages.

Results Circadian oscillation of core clock genes BMAL1, Per2 and Rev-erb α in human macrophages (mdms) demonstrates a functional cellular clock. For the first time we reveal that the inflammatory response is dependent on clock phase, exemplified by altered IL-6 LPS stimulated expression ($p < 0.01$). The critical function of Rev-erb α was confirmed through inhibition of the exaggerated IL-6 response by GSK414112 ($p < 0.01$). Lentiviral knockdown of Rev-erb α expression enhanced the IL-6 LPS response ($p < 0.05$) and attenuated GSK414112's effect. It is known that GSK414112 recruits HDAC3 and NCOR onto Rev-erb α repressing transcription. To define whether Rev-erb α directly interacted with the IL-6 promoter, GSK414112's effect on a series of IL-6 reporter genes was investigated. The full length IL6-luc reporter was repressed by GSK414112 ($p < 0.01$) but mutations to the closely related C/EBP ($p < 0.01$) or 3'AP-1 binding sites ($p < 0.05$) inhibited this effect. ChIP studies confirmed direct interaction of Rev-erb α with the IL-6 promoter. Reporter genes expressing consensus sites for C/EBP and AP-1 showed regulation of these transcription factors by Rev-erb α . Novel Rev-erb α actions were identified through transcriptome profiling of human mdms. SERPINE2, IL-6, PTX3 and MMP-12, all implicated in COPD, are regulated by GSK414112. The analysis also revealed a novel mechanism of action, reverse cholesterol transport, previously implicated in pulmonary inflammation. Luminex analysis on mdms from healthy volunteers and COPD alveolar macrophages revealed that GSK414112 significantly repressed secretion of certain cytokines including IL-6, eotaxin, IL-10, IP-10, G-CSF whilst other cytokines, for example, IL-8, TNF α , MIP 1 α were unaffected.

Conclusion Through Rev-erb α an autonomous cellular clock modifies the macrophage LPS response. Ligands for this nuclear receptor exert anti-inflammatory effects through suppression of target gene transcription and up regulation of the cholesterol efflux pathway, employing a novel transcription factor cross-talk mechanism. This mechanism is effective in suppressing glucocorticoid resistant targets as well as targeting the temporal aspects of inflammation.

T2 BLOCKADE OF INTRAALVEOLAR P55 TNF-RECEPTOR SIGNALLING BY A DOMAIN ANTIBODY DECREASES INFLAMMATION AND OEDEMA IN AN *IN VIVO* MOUSE MODEL OF VENTILATOR-INDUCED LUNG INJURY

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¹S Bertok, ¹M R Wilson, ²P J Morley, ²R de Wildt, ²A Bayliffe, ¹M Takata. ¹Section of Anaesthetics, Pain Medicine and Intensive Care, Imperial College London, Chelsea and Westminster Hospital, London, UK; ²Biopharmaceutical R&D, GlaxoSmithKline, Stevenage, UK

Introduction and Objectives Tumour necrosis factor (TNF) alpha is transiently up-regulated within the alveolar space during ventilator-induced lung injury (VILI). We previously found that the two TNF

receptors play opposing roles during VILI in knock-out mice, with p55 promoting but p75 preventing pulmonary oedema. This suggests that specific blockade of p55 receptor signalling within the alveolar space may be beneficial in VILI. Domain antibodies (dAbs) are the smallest antigen-binding fragments of the IgG molecule, which may have advantages over complete antibodies due to their small size and monovalent binding (mAbs often have agonist activity due to receptor cross-linking). In this study we tested the effects of an intratracheally (i.t.) delivered dAb that binds to and inhibits the murine p55 receptor (Biopharmaceutical R&D, GlaxoSmithKline), on pulmonary oedema and inflammation in mouse models of VILI.

Methods C57BL/6 mice were ventilated with a high-stretch protocol (standardised by plateau pressure at 12.5–13.5 cm H₂O; tidal volume 20–22 ml/kg, PEEP 3 cm H₂O, O₂ with 2–4% CO₂). Mice then received an i.t. bolus of either non-specific 'dummy' dAb or p55-specific dAb (25 μ g in 50 μ l), and were ventilated for up to 4 h (1-hit model). As a 2-hit model, 20 ng LPS were included in the dAb bolus. Development of lung injury was assessed by respiratory elastance and blood gases, and protein level in bronchoalveolar lavage fluid (BALF) at termination. In the 2-hit model, neutrophil infiltration into BALF, the activation state of alveolar macrophages, and neutrophil margination within lung vasculature were all assessed by flow cytometry.

Results High stretch ventilation produced deteriorations in elastance and PO₂ and high BALF protein in both models. Treatment with the p55-specific dAb substantially attenuated all of these changes in the 1-hit model (Abstract T2 Table 1). In the 2-hit model, p55 blockade prevented deteriorations in elastance and oxygenation, and significantly decreased neutrophil margination, intraalveolar neutrophil infiltration and ICAM-1 expression on alveolar macrophages.

Abstract T2 Table 1

	dummy dAb		p55-dAb	
	After instillation	End	After instillation	End
1-hit model				
Elastance (ml/cmH ₂ O/kg)	0.91±0.13	1.07±0.21*	0.91±0.16	0.93±0.18
PO ₂ (mm Hg)	444±72	287±180*	450±26	415±86
BALF protein (mg/ml)	2.9±1.7		1.4±0.4†	
2-hit model				
Elastance (ml/cmH ₂ O/kg)	0.94±0.11	1.08±0.2*	0.88±0.08	0.9±0.11
PO ₂ (mm Hg)	486±21	305±168*	497±16	434±114
BALF protein (mg/ml)	3.0±1.6		2.1±1.5	
Lung neutrophils	2.30±0.57×10 ⁶		1.25±0.34×10 ⁶ †	
BALF neutrophils/ml	1.92±1.69×10 ⁵		0.47±0.41×10 ⁵ †	
ALveolar macrophage ICAM-1 (MFI)	80±20		56±13†	

Mean \pm SD, N=8–10.* $p < 0.05$ vs after instillation.† $p < 0.05$ vs dummy.

Conclusions Use of dAbs to selectively inhibit intra-alveolar p55 TNF receptor signalling may open new therapeutic approaches for ventilated patients with acute lung injury. This study was supported by Biopharmaceutical R&D, GlaxoSmithKline.

T3 TISSUE INHIBITOR OF METALLOPROTEINASE-3 (TIMP3) PROTECTS AGAINST INFLAMMATORY PROCESSES IN INTERSTITIAL LUNG DISEASE (ILD)

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¹M J Heightman, ²H L Booth, ¹G J Laurent, ¹M R Hill, ¹R M McAnulty. ¹University College London, London, UK; ²University College London Hospitals NHS Trust, London, UK

Introduction TIMP3 expression in the lung increases with age and in ILD. TIMP3 binds extracellular matrix (ECM) where it influences