GENETIC AND EPIGENETIC CHARACTERIZATION OF THE SPHINGOSINE-1-PHOSPHATE SIGNALLING SYSTEM IN MACROPHAGES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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LIST OF ABBREVIATIONS

AM	Alveolar macrophage	MCP-1	macrophage chemotactic protein
BAL	Bronchoalveolar lavage	MMP	matrix metalloproteinases
BSP	bisulfite specific PCR	mRNA	Messenger ribonucleic acid
CS	cigarette smoke	NO	nitric oxide
CTGF	Connective tissue growth factor	NTHi	non-typeable H. influenzae.
DNA	Deoxyribonucleic acid	mRNA	Messenger ribonucleic acid
EDTA	Ethylene diamine tetra-acetic acid	PE	Phycoerythrin
EGFR	Epithelial growth factor receptors	PMA	Phorbol 12-myristate 13-acetate
Facs tube	Polystyrene tube (5 ml)	ROS	Reactive oxygen species
FCS	Fetal calf serum	RPMI	Roswell Park Memorial Institute Culture Media
FEV-1	Forced expiratory volume in one second	S1P	Sphingosine 1-phosphate
FVC	Forced vital capacity	S1PR	Sphingosine 1-phosphate receptor
GOLD	Global Strategy for Chronic Obstructive Lung disease	SPHK	Sphingosine kinases
GRO-a	growth related oncogene-alpha	SGPL1	Sphingosine 1-phosphate lyase 1
HDAC	histone deacetylase	SD	Standard deviation
IHC	lmmunohistochemistry	TFBS	Transcription factor binding sites
IL-	Interleukin	TQ	thymoquinone
IP-10	interferon-gamma inducible protein	µg	Microgram
I-TAC	interferon-inducible T-cell alpha-chemoattractant	µI	Microlitre
LPS	Lipopolysaccharide	Wash buffer	0.5% BSA in lsoton II
LTB4	leukotriene B4		
Mab	Monoclonal antibody		

DECLARATION

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LIST OF PUBLICATIONS/MANUSCRIPTS AND ABSTRACTS

Publications/manuscripts related to this research

Hodge G, **Barnawi J**, Jurisevic C, Moffat D, Holmes M, Reynolds P.N, Jersmann H, Hodge S. Lung cancer is associated with decreased expression of perforin, granzyme b and interferon (Genome Bioinformatics Group)-γ by infiltrating lung tissue T cells, natural killer (NK) T-like and NK cells. Clin Exp Immunol. 2014; 178(1):79-85.

Tran H, **Barnawi J**, Roscioli1 E, Hodge G, Reynolds P.N, Pitson S, Jersmann H, Meech R, Haberberger R, Hodge S. Pulmonary macrophage efferocytosis in COPD is suppressed by disturbances to feedback mechanisms in sphingosine-1 phosphate signalling and reversed with FTY720 (manuscript under preparation).

Barnawi J, Tran H, Jersmann H, Pitson S, Roscioli1 E, Hodge G, Meech R, Haberberger R, Hodge S. Potential link between the sphingosine-1-phosphate (S1P) system and defective alveolar macrophage phagocytic function in chronic obstructive pulmonary disease (COPD). (Accepted for publication, Plos One, February 2015).

Barnawi J, Jersmann H, Moffat D, Haberberger R, Hodge S, Meech R.
Differential DNA methylation pattern of sphingosine-1 phosphate receptor 5 in lung macrophages in COPD with/without lung cancer: a potential link to failed efferocytosis. (Submitted to Plos One, July 2015).

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system. (Submitted to Cellular Immunology September 2015; Manuscript Number CIMM-15-266).

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ABSTRACT

Alveolar macrophages from patients with chronic obstructive pulmonary disease (COPD) are defective in their ability to phagocytose apoptotic bronchial epithelial cells (a process termed 'efferocytosis') and bacteria. These defects may contribute to COPD pathogenesis in several ways. Secondary necrosis of uncleared apoptotic material may result in chronic airways inflammation and perpetuation of COPD disease. A reduced alveolar macrophage phagocytic host response to bacteria, especially non-typeable *H influenzae* (NTHi), may contribute to neutrophilic inflammation and NTHi colonization of the lower airway. However, the exact mechanism that leads to the phagocytic dysfunction is still unknown. The sphingosine 1-phosphate (S1P) signalling system is known to regulate macrophage function. Experiments described in Chapter 2 of the thesis therefore applied a novel approach of measuring all S1P signalling system components in alveolar macrophages from COPD patients and healthy controls. Several components of the S1P system, in particular relative mRNA levels for sphingosine kinases *SPHK1* and S1P receptor *S1PR5*, were dysregulated in COPD and were strongly correlated with efferocytosis, suggesting a potential link to the defective alveolar macrophage phagocytic ability in COPD.

Oxidative stress and inflammation have been shown to contribute to many COPD characteristics, such as uncontrolled activation of cell signalling pathways, increased airway epithelial cell apoptosis, and defective alveolar macrophage phagocytic ability. Chapter 3 describes the effect of two models of oxidative stress and inflammation, cigarette smoke (potential oxidative conditions) and lipopolysaccharide (LPS) (potential inflammatory conditions) on components of S1P signalling and on efferocytosis and phagocytosis of NTHi, using a human macrophage cell line *in vitro*. Cigarette smoke and LPS increased the mRNA expression of *SPHK1* and *S1PR5* in macrophages,

extending the results in Chapter 2 and further supporting the potential link between the S1P signalling system and macrophage phagocytic ability. Cigarette smoke decreased the capacity of macrophages to phagocytose apoptotic cells and bacteria. However, LPS reduced phagocytosis of bacteria only. Treatment option for oxidative stress is anti-oxidants and thymoquinone (TQ) is anti-oxidant/anti-inflammatory agent that has been shown to modulate macrophage inflammatory responses and has successfully been trialled in human clinical studies. Chapter 3 further reports that TQ *per se* had a pro-phagocytic effect on macrophage phagocytic ability. TQ also rescued macrophages from the negative effects of cigarette smoke, and to lesser extent LPS, on macrophage efferocytosis and the mRNA expression respectively. In addition, TQ demonstrated a pro-survival effect on bronchial epithelial cells treated with cigarette smoke. The effects on relative mRNA expression of *SPHK1* and *S1PR5* in the cell line were mirrored using acutely isolated alveolar macrophages from COPD patients.

COPD patients are at increased risk for developing lung cancer and there is strong evidence that pulmonary macrophage dysfunction plays an important role in the pathogenesis of both diseases. DNA methylation has been shown to be modified in COPD and lung cancer. However, it unknown whether the change in mRNA expression of the S1P system (Chapter 2) are controlled by epigenetic modifications such as DNA methylation, and whether DNA methylation regulates macrophage efferocytosis. Data presented in Chapter 4 connect epigenetic modulation, mRNA expression and macrophage function. The results indicate that DNA methylation potentially regulates macrophage efferocytosis and is negatively correlated with the mRNA expression of S1P system components, in particular the *S1PR5* receptor, suggesting epigenetic regulation of macrophage efferocytosis in COPD and potentially lung cancer.

In conclusion, the data presented in this thesis have identified the cell- and disease-specific dysregulation of components of the S1P signalling. The combination of *in vitro* studies using a human macrophage cell line and the profiling of acutely isolated lung macrophages from patients and healthy subjects provide powerful evidence for the importance of S1P signalling for efferocytosis function of alveolar macrophages. In addition, it could be demonstrated that oxidative stress might be one cause for the dysregulation and treatment with TQ could rescue effects of the main cause of COPD, cigarette smoke. Furthermore, we could provide for the first time strong evidence for the epigenetic modulation of the *S1PR5* receptor in individual human cells (alveolar macrophages) under pathological conditions (COPD). The data generated as part the thesis are highly valuable for the understanding of macrophage function and S1P under conditions such as COPD. Furthermore, the data also provide possible treatment options for COPD such as anti-oxidants or epigenome modifying agents.