Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Epithelial cytokines and pulmonary allergic inflammation

Clare M Lloyd [1] and Sejal Saglani[1,2]

The triad of epithelial derived cytokines, IL-25, IL-33 and TSLP are important for the initiation and development of pulmonary immune responses to environmental stimuli. Data from experiments using mouse models provide compelling evidence for their involvement in both innate and adaptive immunity to drive type-2 responses, allergic inflammation and airway remodelling. These cytokines are known to be expressed in human lung tissue and immune cells, however their involvement in mediating allergic pulmonary responses in patients is less clear than in murine models of disease. This article focuses on evidence for the role of IL-25, IL-33 and TSLP in human allergic disease and discusses their potential as therapeutic targets for severe asthma.

Addresses
[1] Leukocyte Biology, National Heart & Lung Institute, Imperial College London, UK
[2] Respiratory Paediatrics, Royal Brompton Hospital, London, UK

Corresponding author: Lloyd, Clare M (c.lloyd@imperial.ac.uk)

Current Opinion in Immunology 2015, **34**:52–58

This review comes from a themed issue on **Cytokines**

Edited by **Christopher A Hunter** and **Steven F Ziegler**

http://dx.doi.org/10.1016/j.coi.2015.02.001

Introduction

The pulmonary epithelium is recognised to participate in the initiation and progression of allergic inflammatory pathology. Pulmonary epithelial cells form a barrier against external exposures and expression of a wide range of receptors and molecules enables them to sense and monitor the inhaled environment. Moreover, pulmonary epithelial cells are a rich source of cytokines and chemokines that facilitate the recruitment and activation of a wide range of immune cells that result in the pathophysiological features of the allergic response [1]. The triad of epithelial derived cytokines, IL-25, IL-33 and TSLP, have provoked considerable interest as potential therapeutic targets particularly since the discovery that they elicit the activation and accumulation of innate lymphoid cells to the lung [2]. ILC2 cells produce large amounts of type 2 cytokines even in the absence of an adaptive immune system and are proposed to act in concert with T cells to mediate allergic inflammatory responses [3•]. The fact that the epithelial cytokines are able to promote both innate and adaptive arms of the immune system makes them attractive candidates for novel therapeutics. However, most of the initial data regarding their pulmonary expression and contribution to allergic airways disease has been derived from experiments using mouse models [4]. Evidence from genome wide association studies (GWAS) implicates IL-33 and TSLP in particular in the pathophysiology of allergic diseases making their investigation in human disease especially relevant [5,6]. The wide range of murine studies has allowed formulation of hypotheses regarding the *in vivo* function of IL-25, IL-33 and TSLP. However, evidence for involvement in human disease stems from expression studies using immunohistochemical staining of lung biopsies and *in vitro* functional studies using primary pulmonary cells. We will review the evidence implicating involvement of these epithelial derived cytokines in the development of inflammation in asthmatic patients.

IL-25

Expression of IL-25 has been documented in patients with asthma in a variety of different cell types, including T cells, eosinophils and mast cells as well as lung structural cells, including endothelium and the airway epithelium [7]. Asthmatic patients exhibit cells with the potential to secrete or respond to IL-25 and IL-25$^+$ cells increased in the bronchial submucosa following allergen exposure [7]. These IL-25$^+$ cells were identified as eosinophils, mast cells and endothelial cells whereas epithelial IL-25 expression was not modulated by allergen exposure. Plasma levels of IL-25 are increased in asthmatics compared to controls and levels correlate with lung function [8••]. Additionally, atopic asthmatics exhibit enhanced expression of the IL-25R on eosinophils implying that IL-25 has a role in eosinophil activation in these patients.

Airway epithelial cells constitutively express IL-25 when grown in culture and on exposure to allergens such as HDM, IL-25 protein is rapidly released [9•]. Protease activity was shown to be important for this release, since HDM and other allergens with proteolytic activity enhanced both transcription of IL-25 mRNA and IL-25 protein release via a mechanism involving protease activated receptor2 (PAR2) expressed on the epithelial cell surface [9•].

A potential role for IL-25 in the development of airway remodelling and angiogenesis was highlighted after expression was determined in vascular endothelial cells. Moreover, IL-25 was shown to increase proliferation of endothelial cells and promote growth of microvessels *in vitro* [10]. Interestingly, the number of IL-25$^+$ cells, but not IL-25R$^+$ cells correlated with lung function leading the authors to conclude that IL-25 plays a role in regulating asthma severity. Further evidence for a particular role for IL-25 in lung remodelling stems from studies which show increased expression of pulmonary IL-25 and ILC2s in patients with idiopathic pulmonary fibrosis (IPF), where the authors showed a correlation between IL-25 and levels of extracellular matrix proteins [11].

A recent study outlined a role for IL-25 in viral exacerbations of asthma induced by rhinovirus [12••]. Beale *et al*. used a combination of *in vitro* experiments with rhinovirus exposed human bronchial epithelial cells and an elegant *in vivo* human viral challenge study to show that epithelial IL-25 expression is enhanced in asthmatics compared to controls on exposure to rhinovirus. Blockade of IL-25 in a mouse model of rhinovirus induced allergic exacerbation confirmed a role for IL-25 in the induction of a range of Th2 cytokines, and ameliorated key features of disease, including recruitment of eosinophils, ILC2s and TH2 cells, suggesting this axis is an attractive therapeutic target for viral induced exacerbations [12••].

Asthma is recognised as a heterogeneous disease and there is much interest in the identification of biomarkers that distinguish subsets of patients, particularly if the biomarker predicts response to therapy. Increased bronchial epithelial expression of IL-25 identified a subset of asthmatics that were phenotypically distinct [8••]. The patients were steroid naïve at recruitment, and the IL-25hi subset had greater AHR, enhanced eosinophils in the airways and peripheral circulation, higher serum IgE levels and increased remodelling as measured by basement membrane thickening. In addition, qPCR analysis of bronchial epithelial cells determined greater expression of a key Th2 gene signature (transcripts for periostin, CLCA1 and serpin B2) in patients from the IL-25hi subset who showed improved lung function following treatment with inhaled corticosteroids (ICS). Importantly, plasma IL-25 levels correlated with pulmonary eosinophilic inflammation and lung epithelial IL-25 expression as well as a positive response to ICS, indicating the potential of IL-25 as a biomarker for this subgroup of patients. Interestingly, of the three innate cytokines, only IL-25 epithelial expression was increased in asthmatics, IL-33 and TSLP were similar in asthma and controls.

IL-33

The importance of IL-33 in the pathogenesis of pulmonary allergic disease, specifically asthma, has been highlighted by the repeated identification of the IL-33 and ST2/IL1RL1 (IL-33 receptor) genes as major susceptibility loci in genome wide association studies (GWAS) studies [6,13,14]. IL-33 biology is complex, full length IL-33 released as an alarmin is biologically active, however its bioactivity can be increased ten-fold following processing by inflammatory proteases such as neutrophil elastase and cathepsin G whereas processing by caspases inactivates IL-33 [15]. Murine studies have revealed IL-33 as a key initiator of acute and chronic allergic airways disease [16], with some suggestion that it may be more important than IL-25 [17]. Despite the data from experimental models, there is little evidence of increased pulmonary epithelial expression or release of IL-33 in human asthma. Recent expression studies have not shown increased epithelial IL-33 in bronchial biopsies from adults [8••] or children with asthma [18••]. However, expression in submucosal inflammatory cells was increased in paediatric severe asthma [18••]. In contrast to other cytokines, expression of IL-33 is apparent in both the nucleus and cytoplasm of pulmonary cells, but the mechanism by which it is released remains unclear. Numerous human *in vitro* studies have included stimulation of primary airway epithelial cells with IL-33 to determine its action [19–21]. Only one study has shown IL-33 induction after rhinovirus infection of asthmatic primary bronchial epithelial cells, and an interaction between the culture supernatants and T cells and ILCs resulting in release of type 2 cytokines [22••]. A subset of airway basal cells have been identified in human lung tissue which *in vitro* release IL-33 in response to ATP via purinergic receptor signalling [23]. This population of airway basal cells with an endogenous capacity for pluripotency and IL-33 release are increased in patients with COPD and in mouse models of parainfluenza virus result in long term expression of IL-33. It is tempting to speculate that this cell population could be reprogrammed in the asthmatic airway following viral infection resulting in a population of IL-33hi progenitor cells primed to respond to environmental danger signals with exaggerated IL-33 release. Increased levels of IL-33 were present in induced sputum from children with asthma [24]. However, most studies only demonstrate increased levels of released IL-33 in the asthmatic airway after an acute stimulus such as allergen challenge [25] or viral infection [22••], likely due to the kinetics of IL-33 release which appear to be very rapid. Interestingly, in contrast to the data for IL-25 [8••], there is no convincing evidence to date showing the utility of systemic IL-33 levels as a biomarker of disease in asthma, suggesting only pulmonary levels are significantly altered in allergic disease.

IL-33 exerts its effects through a heterodimeric receptor complex including membrane bound ST2 and IL-1RAcP resulting in the production and release of pro-inflammatory cytokines. However, detailed understanding of the signalling pathways activated by IL-33 is still unclear. A common genetic variation at the IL1RL1 locus was found