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Raised interleukin-17 is immunolocalised to neutrophils in cystic fibrosis lung disease

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ABSTRACT: Interleukin (IL)-17 is pivotal in orchestrating the activity of neutrophils. Neutrophilic inflammation is the dominant pathology in cystic fibrosis (CF) lung disease. We investigated IL-17 protein expression in the lower airway in CF, its cellular immunolocalisation and the effects of IL-17 on CF primary bronchial epithelial cells.

Immunohistochemistry was performed on explanted CF lungs and compared with the non-suppurative condition pulmonary hypertension (PH). Airway lavages and epithelial cultures were generated from explanted CF lungs.

Immunoreactivity for IL-17 was significantly increased in the lower airway epithelium in CF (median 14.1%) compared with PH (2.95%, p=0.0001). The number of cells staining positive for IL-17 in the lower airway mucosa was also increased (64 cells·mm^{-1} compared with 9 cells·mm^{-1} basement membrane, p=0.0005) and included both neutrophils in addition to mononuclear cells. IL-17 was detectable in airway lavages from explanted CF lungs. Treatment of epithelial cultures with IL-17 increased production of IL-8, IL-6 and granulocyte macrophage colony-stimulating factor.

In conclusion, immunoreactive IL-17 is raised in the lower airway of people with CF and localises to both neutrophils and mononuclear cells. IL-17 increases production of pro-neutrophilic mediators by CF epithelial cells, suggesting potential for a positive feedback element in airway inflammation.

KEYWORDS: Cystic fibrosis, cytokines, interleukin-17, neutrophils, T-helper cell type 17, transplantation

Cystic fibrosis (CF) is caused by abnormalities in the CF transmembrane conductance regulator (CFTR) gene and is associated with life-long morbidity and premature mortality, principally from lung disease [1]. The CF airway is characterised by neutrophilic inflammation, retention of mucopurulent secretions and chronic endobronchial infection [1, 2].

The cytokine interleukin (IL)-17 plays a central role in pulmonary host defence by orchestrating the accumulation and associated activity of neutrophils in the bronchoalveolar space [3]. A family of IL-17 cytokines has been described, however IL-17A (referred to as IL-17 onwards), and to a lesser extent IL-17F, are the best characterised [4]. The orchestrating effect of IL-17 on neutrophils is achieved indirectly *via* the local release of neutrophil-mobilising factors, including CXC chemokines from cells resident in the lung [3]. Treatment with IL-17 has also been shown to increase expression of the mucin genes MUC5AC and MUC5B by bronchial epithelial cells *in vitro* [5].

Previous work regarding the source of IL-17 has principally focused on a subset of CD4+ T-helper cells (Th) known as Th17 cells that are distinct from the previously recognised Th1 or Th2 subsets [6]. IL-17 therefore represents a strategic link between acquired and innate immunity [7].

Th17 cells are not the only identified source of IL-17 however; IL-17 is also known to be produced by γδ T-cells and natural killer T-cells [8, 9]. Apart from lymphocytes it has also recently been shown that mast cells express IL-17 in rheumatoid arthritis synovium [10]. Moreover, it has been suggested in human alcoholic liver disease and rodent models of lipopolysaccharide-induced airway inflammation, perinuclear anti-neutrophil cytoplasmic antibodies positive vasculitis and kidney ischaemia-reperfusion injury, that neutrophils themselves are a potential source of IL-17 [11–13]. A recent study of human atherosclerosis identified few IL-17 positive CD3+ cells in plaque specimens. Instead, dual-staining approaches showed the presence of myeloperoxidase positive cells staining positively for IL-17, indicating localisation to neutrophils [14].

IL-17 is therefore increasingly potentially linked to neutrophilic inflammation and mucus excess, two cardinal features of CF lung disease in addition to dysregulation of acquired immunity [5, 6, 15].

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Published human studies of IL-17 in CF are rare but some support the importance of this axis. Increased levels of IL-17 have been found in bronchoalveolar lavage (BAL) of children and adults during infective exacerbations and in the serum of clinically stable adults [16–19]. In addition, people with CF who exhibit robust T-cell responses to *Pseudomonas aeruginosa* manifest more severe lung disease [20]. Such work emphasises the need for further translational studies.

We have tested the hypothesis that increased IL-17 protein expression occurs in advanced CF lung disease. We used airway samples taken from CF lungs removed at the time of transplantation to perform immunohistochemistry and compared findings to a non-suppurative condition, pulmonary hypertension (PH), where the lungs were free of infection. Some of the same CF lungs were also used to generate primary bronchial epithelial cell (PBEC) cultures and airway lavages to measure IL-17. The PBEC cultures were used in proof of concept experiments, which examined the effects of IL-17 stimulation on the production of inflammatory mediators in *ex vivo* PBEC cultures.

METHODS

Ethics and consent

Approval was obtained for this study from the Newcastle and North Tyneside 2 Research Ethics Committee (07/Q0906/47). Informed consent was obtained from all participants at the time of acceptance onto the active lung transplantation list at the Freeman Hospital, Newcastle Upon Tyne, UK.

Patients

Brief details of the 43 study patients who underwent lung transplantation for end-stage lung disease, 35 with CF and eight with PH, including pre-operative sputum microbiology are provided in table 1.

Processing of resected lung tissue

Airway blocks were dissected with the aim of providing intermediate/large airways of 1–5 mm diameter with intact columnar airway epithelia. The blocks were fixed in 10% formalin and embedded in paraffin wax. Sections of 5 μm thickness were cut, mounted and stained with haematoxylin and eosin to check for the presence of appropriate airway epithelia.

Airway lavages

30-mL lavage of PBS was performed on freshly explanted CF lungs. This was achieved by installation of saline into the main or first generation bronchus *via* a 20-mL syringe with a 5-cm quill attached that was then aspirated back into the syringe. The resulting fluid was then promptly centrifuged at $734 \times g$ and the supernatant frozen at -20°C prior to analysis.

Immunohistochemistry for IL-17

Endogenous peroxidase activity was blocked by soaking sections in methanolic hydrogen peroxide. The sections were then washed for 10 min in running tap water. Antigen retrieval was performed in citrate buffer at pH 6 for 5 min in a microwave set at 30% power. The sections were then washed three times in tris-buffered saline (TBS) and blocked with 20% normal horse serum (Vector Laboratories, Peterborough, UK) for 30 min. The primary antibody was a goat immunoglobulin (Ig)G polyclonal affinity purified anti-human IL-17 (R&D Systems, Abingdon, UK). In cross-reactivity testing the manufacturer has found that this reagent shows <1% cross-reactivity with recombinant human (rh)IL-17B, rhIL-17C, rhIL-17D, rhIL-17E and 10% with rhIL-17F. The primary antibody was diluted 1:40 in 3% bovine serum albumin (BSA) and applied for 1 h at room temperature. The sections were then washed twice with TBS and treated with biotinylated horse anti-goat secondary antibody (Vector Laboratories) for 30 min. The sections were washed twice with TBS and treated with the ABC Vectastain Elite kit and DAB (Vector Laboratories) as per the manufacturer's instructions. The sections were finally counter-stained with Carazzi's stain for 1 min and then mounted. Negative controls were performed by omission of the primary antibody and treatment with 3% BSA and isotype negative controls with normal goat Ig (R&D Systems).

Quantification of IL-17 staining

Positive staining was measured in the airway epithelium in five randomly selected, non-overlapping ×40 objective high-power fields for each patient. The observer was blinded to the diagnosis of each patient. Image analysis software was used (Image Pro Plus 4.0; MediaCybernetics, Bethesda, MD, USA) to quantify the staining in terms of mean percentage area of epithelium staining positive and number of positive inflammatory cells per mm of basement membrane in the lamina propria and epithelial compartments, as previously described [21]. Neutrophils were identified by appropriate dimensions and characteristic morphological features such as multilobular nuclei and granular cytoplasm.

Measurement of IL-17 in airway lavages

Airway lavage supernatants were defrosted on ice and then promptly assayed for IL-17 using an ultrasensitive ELISA kit (MesoScale Discovery, Gaithersburg, MD, USA) with a lower limit of detection of 0.2 $pg \cdot mL^{-1}$ as per manufacturer's instructions. The plates were read using the MSD Discovery Workbench analyser and software package (MesoScale Discovery).

Stimulation of PBECs with IL-17

PBECs were isolated and cultured from explanted CF lungs as previously described [22]. Brief details of the patients from whom PBECs were cultured are provided in table 1. First or second passage PBECs were grown to 70–80% confluence in 24-well plates coated with collagen (Vitrogen 100; Cohesion Technologies, Palo Alto, CA, USA) using bronchial epithelial cell growth medium (Lonza, Basel, Switzerland). Cells were rested for 24 h with serum-free resting medium prior to stimulation with 500 μL of resting medium containing 1, 10 or 100 $ng \cdot mL^{-1}$ of IL-17 (R&D Systems).

Measurement of IL-8, IL-6 and granulocyte-macrophage colony-stimulating factor in culture supernatants

The contents of each well was removed by pipette after 24 h, centrifuged at $183 \times g$ for 2 min and the supernatant immediately frozen at -80°C prior to analysis. A multiplex ELISA was then performed as per manufacturer's instructions (Meso Scale Discovery).

Statistical analysis

Non-parametric comparisons were made using the Mann–Whitney test for staining between groups and the Wilcoxon signed rank test for PBEC stimulation experiments. A p-value <0.05 was considered to be statistically significant.

TABLE 1 Details of patients undergoing lung transplantation

Patient number	Diagnosis	Pre-operative sputum microbiology	IHC	AL	PBEC culture
1	CF	*P. aeruginosa*	+	+	-
2	CF	*P. aeruginos, S. aureus*	+	+	-
3	CF	*P. aeruginosa, S. aureus, S. maltophilia, A. fumigatus*	+	-	-
4	CF	*P. aeruginosa*, *S. aureus*	+	-	-
5	CF	*P. aeruginosa*, *A. fumigatus*	+	-	-
6	CF	*P. aeruginosa*, *S. maltophilia*	+	+	-
7	CF	*P. aeruginosa*, methicillin-resistant *S. aureus*	+	+	-
8	CF	*P. aeruginosa*, *B. vietnamiensis*	+	+	-
9	CF	*P. aeruginosa*	+	+	+
10	CF	*S. aspiospermum*, *S. aureus, Alcaligines* sp.	+	+	+
11	CF	*P. aeruginosa*	+	-	-
12	CF	*P. aeruginosa, S. aspiospermum*	+	-	-
13	CF	*P. aeruginosa, A. fumigatus*	+	+	+
14	CF	*P. aeruginosa*	+	-	-
15	CF	*P. aeruginosa*	+	+	-
16	CF	*P. aeruginosa*	+	+	+
17	CF	*P. aeruginosa, S. aureus*	+	+	-
18	CF	*P. aeruginosa, S. aureus*	+	+	-
19	CF	*P. aeruginosa*	+	-	
20	CF	*S. maltophilia, Acinetobacter* sp.	-	+	+
21	CF	*P. aeruginosa*	-	+	+
22	CF	*P. aeruginosa*	-	+	+
23	CF	*P. aeruginosa, A. fumigatus*	-	+	+
24	CF	*P. aeruginosa, A. fumigatus*	-	+	+
25	CF	*B. multivorans*, *A. fumigatus*	-	+	-
26	CF	*P. aeruginosa*	-	+	-
27	CF	*P. aeruginosa A. fumigatus*	-	+	-
28	CF	*P. aeruginosa*	-	+	-
29	CF	*P. aeruginosa*	-	+	-
30	CF	*P. aeruginosa*, *A. fumigatus*	-	+	-
31	CF	*P. aeruginosa*, *M. gordonae*	-	+	-
32	CF	*P. aeruginosa*	-	+	-
33	CF	*B. multivorans*	-	+	-
34	CF	*P. aeruginosa*, *S. maltophilia*	-	+	-
35	CF	*P. aeruginosa*, *A. fumigatus*	-	+	-
36	PH	Negative	+		-
37	PH	Negative	+		-
38	PH	Negative	+		-
39	PH	Negative	+		-
40	PH	Negative	+		-
41	PH	Negative	+		-
42	PH	Negative	+		-
43	PH	Negative	+		-

IHC: immunohistochemistry; AL: airway lavage; PBEC: primary bronchial epithelial cell; CF: cystic fibrosis; PH: pulmonary hypertension; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *A. fumigatus*: *Aspergillus fumigatus*; *B. vietnamiensis*: *Burkholderia vietnamiensis*; *S. aspiospermum*: *Scedosporium aspiospermum*; *B. multivorans*: *Burkholderia multivorans*; *M. gordonae*: *Mycobacterium gordonae*.

RESULTS

Immunoreactivity for IL-17 is increased in the lower airway epithelium of people with CF

The mean percentage area of lower airway epithelium positive for IL-17 in each individual is displayed in figure 1. Staining for IL-17 was significantly higher in the CF group, median 14.1%, compared with PH (3.0%; p=0.0001). Figure 2 shows representative IL-17 staining in an explanted CF and PH lung and negative control.

IL-17 positive cells are increased in the airway mucosa in CF and include neutrophils

The number of IL-17 positive cells in the mucosa was significantly increased in the CF group, median 64 cells·mm^{-1}

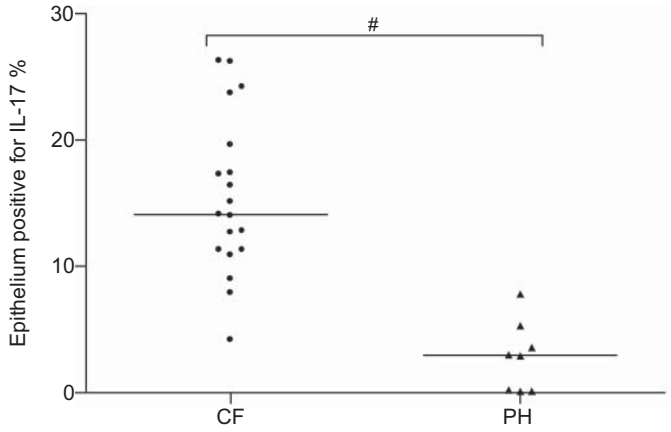

FIGURE 1. Percentage of epithelium staining positive for interleukin (IL)-17. Each symbol represents an individual patient and represents the mean from five high-power fields. Horizontal bar indicates the median for each group. CF: cystic fibrosis; PH: pulmonary hypertension. #: $p<0.0001$.

of basement membrane, and included both neutrophils and mononuclear cells, compared with PH, 9 cells·mm^{-1} basement membrane ($p=0.0005$). The mean number of positive cells per mm of basement membrane in each individual is displayed in figure 3. In the CF group substantial numbers of inflammatory cells staining positive for IL-17 were found in the epithelium, of note this frequently included neutrophils (table 2 and fig. 4). In the PH group, IL-17 positive inflammatory cells were absent from the epithelium (table 2).

Stimulation of PBECs isolated from people with CF with IL-17 increases production of the pro-neutrophilic mediators IL-8, IL-6 and granulocyte-macrophage colony-stimulating factor

The lower airway epithelium is the predominant site of neutrophilic inflammation in CF lung disease [2]. Airway epithelial cells are increasingly recognised to operate as ''effector'' cells that produce a wide range of inflammatory cytokines and growth factors [23]. The cytokines IL-8, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are important in neutrophil activation, recruitment and longevity [24]. We therefore investigated the effects of IL-17 on the production of these mediators by *ex vivo* cultures of PBECs from people with CF.

There was a significant increase in IL-8 production from control conditions following stimulation with 1 ($p=0.033$), 10 ($p=0.009$) and 100 ng·mL^{-1} ($p=0.009$) of IL-17 (fig. 5a) ($n=9$ individual patient donors). There was also a statistically significant increase in IL-6 production (fig. 5b) from control following stimulation with 10 and 100 ng·mL^{-1} of IL-17A ($p=0.009$). There was a clear trend towards increased production of IL-6 following stimulation with 1 ng·mL^{-1} of IL-17 although this was not statistically significant ($p=0.076$). Increase in production of GM-CSF (fig. 5c) was statistically significant following stimulation with IL-17 100 ng·mL^{-1} only ($p=0.013$). There was a nonsignificant trend towards increased levels following stimulation with IL-17 at 1 and 10 ng·mL^{-1} ($p=0.407$ and 0.193, respectively).

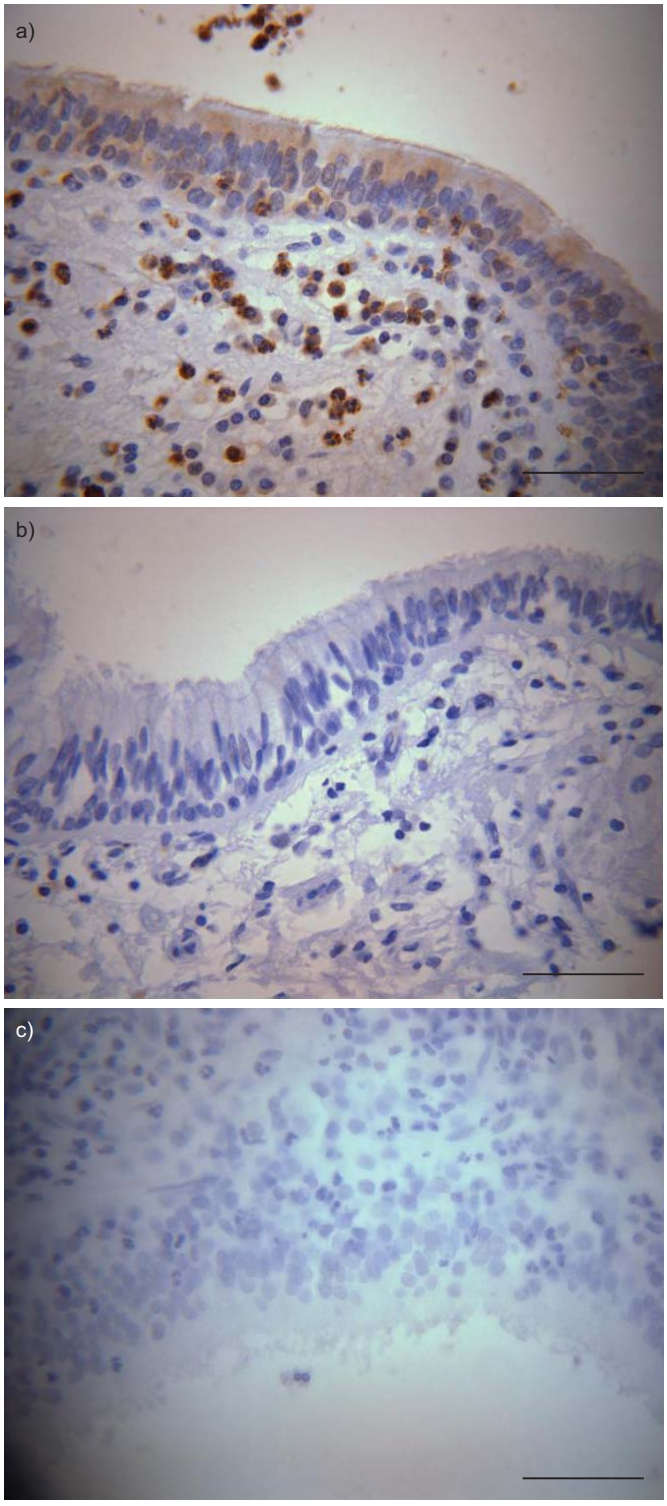

FIGURE 2. Representative immunohistochemistry for interleukin-17 of an explanted lung from a patient with a) cystic fibrosis and b) pulmonary hypertension. c) Negative control (no primary antibody added). Scale bars=50 μm.

IL-17 is detectable in airway lavages from explanted CF lungs

IL-17 was detectable in all of the airway lavages from explanted CF lungs (fig. 6). The median concentration was

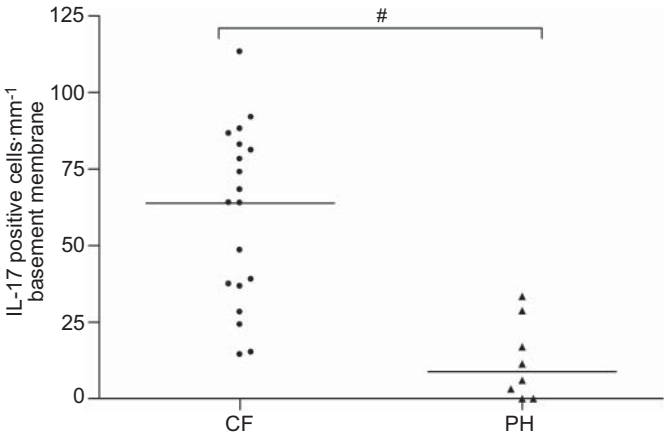

FIGURE 3. Number of cells positive for interleukin (IL)-17 in the lower airway mucosa. Each symbol represents an individual patient and represents the mean from five high-power fields. Horizontal bar indicates the median for each group. CF: cystic fibrosis; PH: pulmonary hypertension. #: p=0.0005.

56.9 pg·mL^{-1} when corrected for dilution, *i.e.* raw data multiplied by the lavage volume used.

DISCUSSION

In this study we have demonstrated by immunohistochemistry that IL-17 is expressed and raised in the lower airway of people with advanced CF lung disease compared with the non-suppurative condition PH. IL-17 was also detectable in airway lavage fluid from explanted CF lungs. There were substantially increased numbers of inflammatory cells staining positive for IL-17 present in the lower airway mucosa of people with CF. Some of these were mononuclear cells in keeping with the literature, indicating that Th17 cells are a source of IL-17 [7]. However, we also consistently identified abundant IL-17 positive neutrophils, a new finding which we suggest may be significant in CF lung disease. We chose PH as a disease comparator because it represents a non-suppurative condition and the tissue is readily available in our centre. A potentially important disease group for future comparison would be non-CF bronchiectasis.

As far as we are aware there are no previous descriptions showing that IL-17 protein is localised to neutrophils in the human lung, with a strong emphasis on Th17 biology in the current literature. However, IL-17 staining has been described previously in neutrophils in the context of human liver disease and atherosclerosis and in the lungs of mice [11, 12]. Human studies of oral inflammation have also shown that neutrophils may be an important source of IL-17 protein [25]. In a study of 15 volunteer blood donors and 20 patients with oral inflammation (*e.g.* dental abscesses) a 37-kDa band identified as IL-17A on Western blots was demonstrable in peripheral blood neutrophils and mononuclear cells. Densitometry suggested that higher levels of IL-17A protein could be detected in the neutrophils of patients compared with neutrophils from control subjects [25].

Elegant work, only achievable in a mouse setting, recently showed that renal ischaemia–reperfusion injury is IL-17-dependent and that IL-17 is produced by neutrophils [13].

TABLE 2 Number of interleukin (IL)-17 positive cells in the mucosa, epithelium and lamina propria in lungs explanted from people with cystic fibrosis (CF) and pulmonary hypertension (PH)

	CF	PH
IL-17 positive cells·mm^{-1} basement membrane		
Mucosa	63.9 (37.0–81.9)#	8.8 (2.4–19.9)
Epithelium	17.6 (8.5–23.8)	0
Lamina propria	41.1 (19.0–59.9)¶	8.8 (2.4–19.9)

Data are presented as median (interquartile range). #: p=0.0005; ¶: p=0.032.

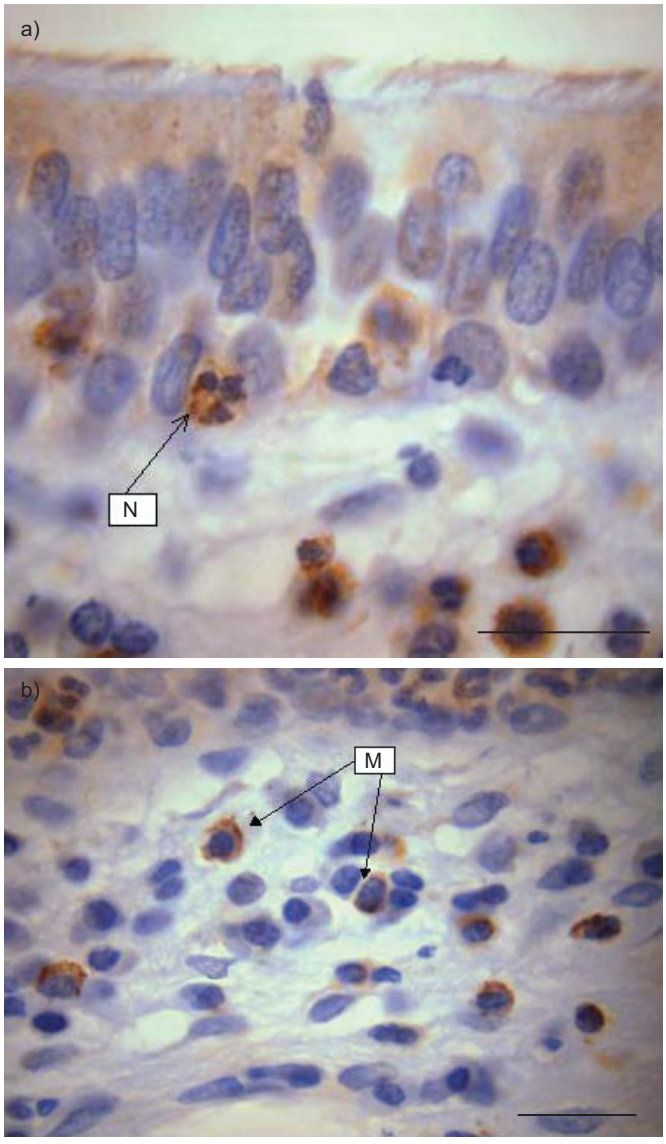

FIGURE 4. a) Example of neutrophil (N) staining positive for interleukin (IL)-17 in the epithelium of an explanted lung from a patient with cystic fibrosis. b) Example of mononuclear cells (M) staining positive for IL-17 in the explanted lung of a patient with cystic fibrosis. Scale bars=20 μm.

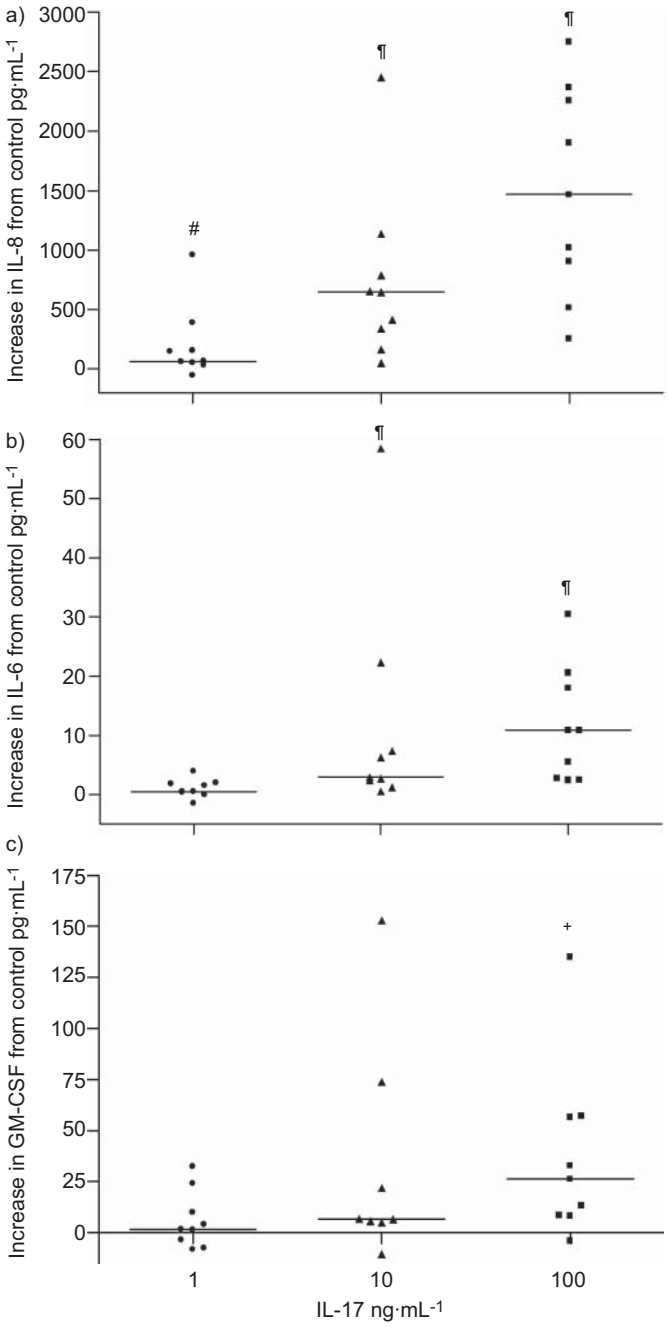

FIGURE 5. Increase in production of a) interleukin (IL)-8 (median basal production 242 pg·mL^{-1}), b) IL-6 (median basal production 2.04 pg·mL^{-1}), and c) granulocyte-macrophage colony-stimulating factor (GM-CSF; median basal production 8.08 pg·mL^{-1}) by primary bronchial epithelial cells from patients with cystic fibrosis from control following stimulation with 1, 10 and 100 ng·mL^{-1} IL-17. Each symbol represents an individual patient and is the mean of three replicate experiments (n=9 individual patient donors). Horizontal bars indicate median for each group. $^{\#}$: p=0.033; ¶: p=0.009; $^{+}$: p=0.013.

This work used IL-17 knockouts and IL-17 production assays, as well as bone marrow transplantation to produce chimeric mice. A series of experiments showed that kidney damage was IL-17-mediated and that this was produced by bone marrow-derived neutrophils. Hence, protection from kidney injury was shown in IL-17-/- mice and this was reversed following

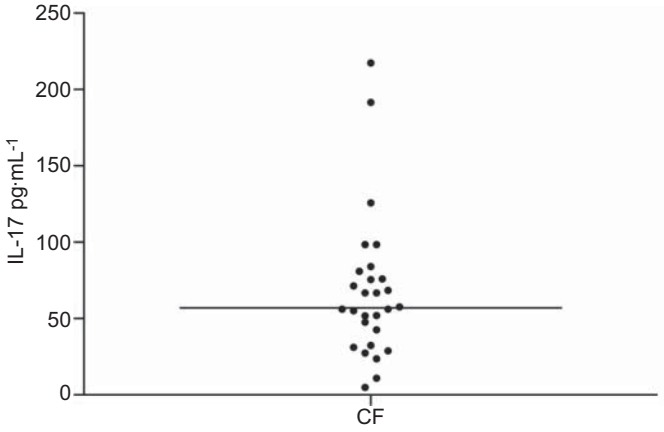

FIGURE 6. Interleukin (IL)-17 in airway lavage fluid from explanted cystic fibrosis (CF) lungs. Each symbol represents an individual patient and concentrations account for a dilution factor of 30. The horizontal line represents the median (56.9 pg·mL^{-1}).

transfer of wildtype neutrophils, but not IL-17-/- neutrophils. Where reconstituted the injury was in turn attenuated by antibody neutralisation of IL-17. It should be noted, however, that as far as we are aware neutrophils were not sorted and assessed for IL-17 transcripts in this study.

Our finding that airway wall neutrophils stain positively for IL-17 does not necessarily mean that they are the source of the ligand. Neutrophils express the IL-17 receptor and can bind IL-17 [26]. However, this would appear to be an unlikely sole explanation due to the diffuse intracellular staining that we identified. If IL-17 is adherent to receptors on neutrophils this may also represent a biologically significant reservoir that is potentially released into the microenvironment following neutrophil necrosis [24, 27].

If neutrophils are a source of IL-17, as evidenced by our current human lung work, the aforementioned human liver, oral inflammation and atherosclerosis studies and in more detailed animal model work, this suggests that IL-17 mediated injury may have a positive feedback element [11–13]. IL-17 causes neutrophil recruitment, which may be potential sources of this key cytokine [12]. IL-17 also has a modulatory post-transcriptional effect on IL-8 and IL-6 responses by epithelial cells in addition to stimulatory effects [28].

A potential for positive feedback was further suggested by our proof of concept experiments. In these we assessed the effect of IL-17 stimulation on CF airway epithelial cells. Our chosen approach was to perform technically challenging, primary cultures generated from some of the CF lungs that were used in IL-17 immunolocalisation. This strategy was used in order to maintain translational focus on a patient-derived *ex vivo* experimental model. IL-17 is known to exert effects on neutrophil accumulation and activation in the bronchoalveolar space indirectly *via* the local release of neutrophil-mobilising factors [3, 6]. We therefore investigated the effects of treatment with IL-17 on the production of the key pro-neutrophilic cytokines and chemokines IL-8, IL-6 and GM-CSF by PBEC cultures from people with CF. This showed that stimulation of PBECs with IL-17 resulted in significant increases in the release

of these key mediators. MCALLISTER *et al.* [16] detected IL-17 at ~100 pg·mL^{-1} in BAL fluid from people with CF and we found a median of ~50 pg·mL^{-1} in airway lavages. It may be argued that stimuli >1–2 ng·mL^{-1} IL-17 are therefore supraphysiological, in our defence however these were proof of concept experiments and concentrations of cytokine at the immunological synapse *in vivo* may be far higher [16].

IL-8 is a potent chemoattractant and activator of neutrophils and is the dominant neutrophil chemokine in the sputum of people with CF during an infective exacerbation [16, 29]. Substantial literature also shows that IL-8 is involved in airway angiogenic structural remodelling [30]. Stimulation with IL-17 resulted in a significant increase in IL-8 production in all of our culture experiments. This data is therefore consistent with a potential contributory role in neutrophilic airway inflammation and remodelling in CF. The growth factor GM-CSF displays pleiotropic effects on neutrophil proliferation, maturation, activation and inhibition of apoptosis [31]. Our finding that treatment of PBECs with IL-17 caused increased GM-CSF production is also therefore consistent with a broad association between IL-17, epithelial cells and neutrophilic inflammation in CF. Furthermore, in the context of myocarditis, there is evidence to suggest that GM-CSF plays a role in the generation and maintenance of Th17 cells *via* regulation of IL-23 and IL-6 *in vivo* [32].

Production of IL-6 was also increased significantly following treatment with 10 and 100 ng·mL^{-1} IL-17 with a clear but nonsignificant trend towards increased levels at 1 ng·mL^{-1}. IL-6 plays a key role in the acute phase inflammatory response [33]. Furthermore, IL-6 induces IL-17 production from human central memory CD4+ T-cells [34]. Together with our finding that neutrophils themselves are a source of IL-17, the increase in IL-6 production by PBECs following IL-17 treatment represents a further potential aspect of a positive feedback loop and augmentation of IL-17 responses. Furthermore, it has recently been shown that following stimulation with IL-17, IL-6 production by bronchial epithelial cells with intact CFTR function is enhanced when co-cultured with basophils compared with when cultured alone [35].

It was apparent that there was a high degree of variability in the production of IL-8, GM-CSF and IL-6 in PBECs isolated from different individuals. This reflects previously described inherent biological variability in chemokine and cytokine production, and is in keeping with the experiences of other researchers with PBECs [36]. Interestingly, the findings in our *ex vivo* system resonate with the biological variability seen in the human CF lung disease phenotype, although all of our patients ultimately developed advanced lung disease [1].

Other findings in the published literature pertinent to a role for IL-17 in CF lung disease include the reports that immortalised airway epithelial cells that do not express functional CFTR upregulate their innate immune responses following stimulation with IL-17. In particular, nucleotide-binding oligomerisation domain 1 is increased and IL-17 also modulates bicarbonate secretion in normal airway epithelial cells [37, 38].

Although our study includes potentially significant observations, it has limitations. Our work was clearly restricted to a study of advanced CF lung disease, utilising our well characterised and substantive tissue archive. However, this precluded any meaningful exploration of clinical associations and did not include any individuals with less severe lung disease. Further work, in both earlier stages and less severe phenotypes of CF lung disease, is therefore warranted.

Our suggestion that IL-17 is produced by airway neutrophils was achieved using unambiguous, immunolocalisation using an affinity purified, specific antibody [39]. We clearly showed IL-17 positive, intra-epithelial neutrophils. Our results do not, however, indicate what the levels of local IL-17 release are at this precise intra-epithelial site. In particular, concentrations at the immunological interface between neutrophils, lymphocytes and epithelial cells are not known.

Our study describes elevated IL-17 in the airways of patients with advanced CF lung disease and the first description that neutrophils are a potential source of this key cytokine in human airways. This should stimulate further translational work as well as other approaches including appropriate animal models. We feel that such work may be particularly important in CF, but will also be relevant in other lung pathophysiologies involving neutrophil biology.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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REFERENCES

1 O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet* 2009; 373: 1891–1904.
2 Hubeau C, Lorenzato M, Couetil JP, *et al.* Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa. *Clin Exp Immunol* 2001; 124: 69–76.
3 Lindén A, Laan M, Anderson GP. Neutrophils, interleukin-17A and lung disease. *Eur Respir J* 2005; 25: 159–172.
4 Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nature Rev Immunol* 2009; 9: 556–567.
5 Chen Y, Thai P, Zhao YH, *et al.* Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003; 278: 17036–17043.
6 Aujla SJ, Dubin PJ, Kolls JK. Interleukin-17 in pulmonary host defense. *Exp Lung Res* 2007; 33: 507–518.
7 Glader P, Smith ME, Malmhall C, *et al.* Interleukin-17-producing T helper cells and Th17 cytokines in human airways exposed to endotoxin. *Eur Respir J* 2010; 36: 1155–1164.
8 Michel ML, Keller AC, Paget C, *et al.* Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia. *J Exp Med* 2007; 204: 995–1001.
9 Roark CL, Simonian PL, Fontenot AP, *et al.* γδ T cells: an important source of IL-17. *Curr Opin Immunol* 2008; 20: 353–357.

10 Hueber AJ, Asquith DL, Miller AM, *et al.* Mast cells express IL-17A in rheumatoid arthritis synovium. *J Immunol* 2010; 184: 3336–3340.
11 Lemmers A, Moreno C, Gustot T, *et al.* The interleukin-17 pathway is involved in human alcoholic liver disease. *Hepatology* 2009; 49: 646–657.
12 Ferretti S, Bonneau O, Dubois GR, *et al.* IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. *J Immunol* 2003; 170: 2106–2112.
13 Li L, Huang L, Vergis AL, *et al.* IL-17 produced by neutrophils regulates IFN-γ-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. *J Clin Invest* 2010; 120: 331–342.
14 de Boer OJ, van der Meer JJ, Teeling P, *et al.* Differential expression of interleukin-17 family cytokines in intact and complicated human atherosclerotic plaques. *J Pathol* 2010; 220: 499–508.
15 Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* 2007; 8: 345–350.
16 McAllister F, Henry A, Kreindler JL, *et al.* Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-α and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. *J Immunol* 2005; 175: 404–412.
17 Dubin PJ, McAllister F, Kolls JK. Is cystic fibrosis a TH17 disease? *Inflamm Res* 2007; 56: 221–227.
18 Aujla SJ, Chan YR, Zheng M, *et al.* IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat Med* 2008; 14: 275–281.
19 Dufresne V, Knoop C, Van Muylem A, *et al.* Effect of systemic inflammation on inspiratory and limb muscle strength and bulk in cystic fibrosis. *Am J Respir Crit Care Med* 2009; 180: 153–158.
20 Winnie GB, Cowan RG. Respiratory tract colonization with *Pseudomonas aeruginosa* in cystic fibrosis: correlations between anti-*Pseudomonas aeruginosa* antibody levels and pulmonary function. *Pediatric pulmonology* 1991; 10: 92–100.
21 Ward C, Forrest IA, Murphy DM, *et al.* Phenotype of airway epithelial cells suggests epithelial to mesenchymal cell transition in clinically stable lung transplant recipients. *Thorax* 2005; 60: 865–871.
22 Brodlie M, McKean MC, Johnson GE, *et al.* Primary bronchial epithelial cell culture from explanted cystic fibrosis lungs. *Exp Lung Res* 2010; 36: 101–110.
23 Ward C, Eger K, Diboll J, *et al.* Bronchial epithelial cells cultured from clinically stable lung allograft patients promote the development of macrophages from monocytes rather than dendritic cells. *Thorax* 2009; 64: 430–435.
24 Watt AP, Brown V, Courtney J, *et al.* Neutrophil apoptosis, proinflammatory mediators and cell counts in bronchiectasis. *Thorax* 2004; 59: 231–236.
25 Garley M, Jablonska E. Chosen IL-17 family proteins in neutrophils of patients with oral inflammation. *Adv Med Sci* 2008; 53: 326–330.
26 Ye P, Rodriguez FH, Kanaly S, *et al.* Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001; 194: 519–527.
27 Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. *Am J Respir Crit Care Med* 1999; 160: S5–S11.
28 van den Berg A, Kuiper M, Snoek M, *et al.* Interleukin-17 induces hyperresponsive interleukin-8 and interleukin-6 production to tumor necrosis factor-α in structural lung cells. *Am J Respir Cell Mol Biol* 2005; 33: 97–104.
29 Standiford TJ, Kunkel SL, Strieter RM. Interleukin-8: a major mediator of acute pulmonary inflammation. *Reg Immunol* 1993; 5: 134–141.
30 Walters EH, Reid D, Soltani A, *et al.* Angiogenesis: a potentially critical part of remodelling in chronic airway diseases? *Pharmacol Ther* 2008; 118: 128–137.
31 Barreda DR, Hanington PC, Belosevic M. Regulation of myeloid development and function by colony stimulating factors. *Dev Comp Immunol* 2004; 28: 509–554.
32 Sonderegger I, Iezzi G, Maier R, *et al.* GM-CSF mediates autoimmunity by enhancing IL-6-dependent Th17 cell development and survival. *J Exp Med* 2008; 205: 2281–2294.
33 Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006; 8: Suppl. 2, S3.
34 Yang L, Anderson DE, Baecher-Allan C, *et al.* IL-21 and TGF-β are required for differentiation of human T(H)17 cells. *Nature* 2008; 454: 350–352.
35 Wong CK, Cao J, Yin YB, *et al.* Interleukin-17A activation on bronchial epithelium and basophils: a novel inflammatory mechanism. *Eur Respir J* 2010; 35: 883–893.
36 Becker MN, Sauer MS, Muhlebach MS, *et al.* Cytokine secretion by cystic fibrosis airway epithelial cells. *Am J Respir Crit Care Med* 2004; 169: 645–653.
37 Roussel L, Rousseau S. IL-17 primes airway epithelial cells lacking functional Cystic Fibrosis Transmembrane conductance Regulator (CFTR) to increase NOD1 responses. *Biochem Biophys Res Commun* 2009; 391: 505–509.
38 Kreindler JL, Bertrand CA, Lee RJ, *et al.* Interleukin-17A induces bicarbonate secretion in normal human bronchial epithelial cells. *Am J Physiol* 2009; 296: L257–L266.
39 Le Gouvello S, Bastuji-Garin S, Aloulou N, *et al.* High prevalence of Foxp3 and IL17 in MMR-proficient colorectal carcinomas. *Gut* 2008; 57: 772–779.