interleukin (IL)-33 and their production of type-2 cytokines (including IL-5, IL-9, IL-13). ILC2 have a variety of roles in lung inflammation and repair. Their interaction with innate immune cells has been shown, however their influence on the adaptive immune system remains unknown. Given the important roles of CD4 T helper (Th) cells in the lung and in conditions such as asthma, it is vital to determine whether ILC2 can influence their functions.

Aim To determine the interactions of lung-derived ILC2 cells on naïve Th functions.

Method BALB/c mice were treated with IL-33 intranasally for 5 days and ILC2 cells were sorted using fluorescence-activated cell sorting (FACS). Naïve Th cells were sorted by FACS from ST2 knockout mice (lacking the IL-33 receptor). Cells were co-cultured in the presence of anti-CD3 and anti-CD28 antibody for 72 hours. Intracellular cytokines were determined by FACS following phorbol 12-myristate 13-acetate (PMA) and ionomycin stimulation. Th cells were differentiated from ILC2 by their expression of CD4 and lack of ST2.

Results Naïve Th cells expression of type-2 cytokines increases significantly when cultured with ILC2 cells (Fig.1). Th cell IL-4 expression increases 3-fold in co-culture, whilst IL-5 and IL-13 expression are enhanced 30- and nearly 20-fold, respectively. This effect is completely abrogated when cells are separated with a semi-porous membrane. Furthermore, ILC2 are able to enhance T cell responses *in vivo* in the lungs of BALB/c mice.

Discussion These data demonstrate that ILC2 are able to drive a Th2-phenotype in naïve Th cells directly. Furthermore, this effect is contact-dependent. These data demonstrate for the first time that ILC2 are capable of driving type-2 immune responses by influencing Th cell responses and hence provide an important link between lung innate and adaptive immune responses and a possible novel mechanism for their role in asthma.
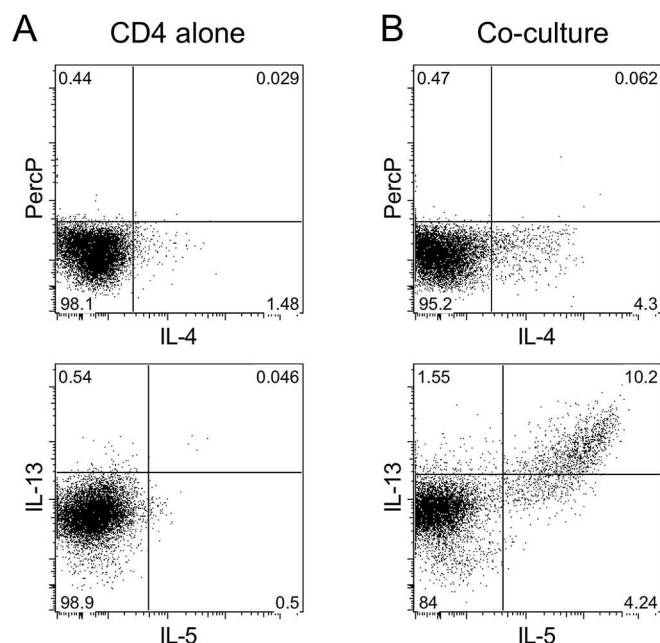
Abstract S21 Figure 1. Th cells express type-2 cytokines when co-cultured with ILC2. Naïve Th cells (CD4$^+$CD44lo) were sorted by FACS from ST2 knockout mice and were cultured alone (A) or with ILC2 (Lin*CD45$^+$ICOS$^+$ST2$^+$) sorted from the lungs of IL-33-treated mice (B) for 72 hours in the presence of anti-CD3/CD28. Th cells intracellular cytokines were determined by FACS following 4 hours stimulation with PMA/ionomycin. CD4 cells were identified by presence of CD4 and lack of ST2.

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Introduction and Objectives Evidence is accumulating for the presence of bacteria in the airways and the existence of a commensal airway microbiome. Molecular techniques reveal complex microbial assemblages in bronchiectasis and COPD, but little is known about the airway microbiome in asthma or in health. Immunopathology in asthma may be driven by bacteria or fungi or even by chronic viral persistence.

We undertook an analysis of the entire respiratory microbial metagenome in airway samples from carefully phenotyped subjects across a spectrum of asthma and health.

Methods 55 subjects (9 mild asthmatics, 16 moderate asthmatics, 15 with severe neutrophilic asthma without bronchiectasis and 15 healthy controls) underwent detailed clinical and immunological phenotyping, sputum induction, and bronchoscopy during periods of clinical stability. Protected bronchoalveolar lavage (BAL) and induced sputum were analysed by whole genome shot-gun sequencing for RNA and DNA from bacterial, viral and fungal genomes. Data were analysed using the Virus-Hunter analysis pipeline.

Results We found no evidence of novel viral species or of persistent viral infection. Protected BAL samples typically contained 500–5000 bacterial reads for organisms typical of the oral cavity or upper respiratory tract, consistent with microaspiration rather than a distinct airway microbiome. Bacterial abundance was not increased in asthma. Hierarchical cluster analysis revealed no general association between disease and the presence of bacteria, with the exception of two individuals with severe neutrophilic asthma in whom single pathogenic species were detected with high abundance. In the first subject, chronic *Haemophilus influenzae* infection was present, correlating with standard culture and a striking Th17 cell response in BAL. Specific antibiotic treatment resulted in a dramatic clinical improvement. In the second individual *Tropheryma whipplei* was present, and correlated with the presence of foamy macrophages and a deficiency of BAL Th17 cells.

Conclusions: The data from this study argue against the existence of a distinct airway microbiome in health or in asthma, but rather that lung microbes are a result of microaspiration. Conversely, in specific cases chronic low grade infection may drive immunopathology in asthma. It remains to be seen whether very severe forms of asthma have a more prominent microbiome.

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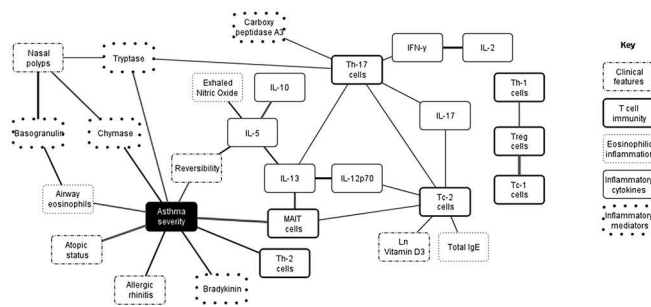
Abstract S23 Figure 1. Bayesian Network Analysis showing statistically significant relationships between clinical and immunological parameters measured in the study. The strength of the interactions (as Euclidean distance) is shown by line weight. Figure 1. Bayesian Network Analysis showing statistically significant relationships between clinical and immunological parameters measured in the study. The strength of the interactions (as Euclidean distance) is shown by line weight.

Introduction and Objectives Asthma is a complex disease involving many cell types including CD4+ and CD8+ T cells, eosinophils, basophils and mast cells, and their soluble mediators. Poor understanding of disease heterogeneity and mechanisms underlying distinct clinico-pathological endotypes limits progress.

We hypothesised that identification of distinct multidimensional endotypes using combined clinical and pathological parameters would enable a better understanding of asthma mechanisms and phenotypes. We undertook to analyse a range of cell types and mediators across a wide clinical spectrum of asthma and health with application of a novel analytical approach: topological data analysis (TDA).

Methods 76 adult subjects underwent extensive phenotyping, using clinical assessment, lung function, methacholine challenge, sputum induction, phlebotomy, and bronchoscopy. Samples were analysed for measurement of CD4+, CD8+ and invariant T cell subsets, of T cell cytokines, of mast cell and basophil mediators, bradykinin and vitamin D, using 9-colour flow-cytometry, RT-PCR, multiplex ELISA and mass spectrometry. We used Bayesian network analysis (BNA) to define the inter-connectivity between the key biological parameters investigated and TDA to identify novel multi-dimensional endotypes of asthma.

Results We generated a Bayesian belief network using 37 key parameters on a subset of 62 subjects with the most complete data (figure), revealing asthma severity as a multidimensional feature, influenced by a wide variety of parameters, including Th2 inflammation, mast cells and basophils.

A TDA network generated using only pathological features revealed distinct pathological endotypes including i) mast cell / basophil mediated ii) severe, obese, high bradykinin iii) minimal inflammation, without allergic rhinitis iv) Th2 mediated, milder disease. Significant differences were observed between clusters in carboxypeptidase, bradykinin, basogranulin, Th2 cells, and IL-4,-5,-12p70.

A TDA network generated using only clinical features revealed a clear dichotomy between steroid responsive and steroid refractory disease. Differences were observed between clinical groups in tryptase, chymase, eosinophils and Th2 and mucosal associated invariant T (MAIT) cells.

Conclusions This study highlights the diversity of aberrant immune responses associated with different clinical and pathological phenotypes of asthma. Attention should focus on further defining these distinct subtypes using such unbiased techniques and characterising under-researched pathways such as CD8+ T cells, basophils and bradykinin.

Clinical trials in COPD

S24 | **A SELF-MANAGEMENT PROGRAMME OF ACTIVITY, COPING AND EDUCATION (SPACE) FOR COPD: A RANDOMISED NON-INFERIORITY TRIAL IN A PULMONARY REHABILITATION POPULATION**

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Introduction Self-managed programmes for patients with COPD offer an alternative to conventional pulmonary rehabilitation (PR). Previously reported self management (SM) programmes either offer short education sessions or action plans or are long intensive programme with a high level of support which are more comprehensive than tradition UK PR programmes. There is limited data reported on self managed approaches to PR which offer minimal support for education, skills acquisition and exercise in comparison to PR in the UK. We have recently developed a SM programme for patients with COPD (SPACE for COPD) and have completed studies in primary care[1]. The demand for PR is high but the service is compromised by limited access and drop out rates. Therefore the aim of this study was to determine if SPACE for COPD is noninferior to (as good as) PR for patients with COPD over 7 weeks.

Methods 287 patients (187 male: mean (SD) age 67 (9) yrs; FEV_1 1.25 (0.55); BMI 27.63 (6.22) kg/m^2) with COPD were consented and randomised to either SPACE for COPD or PR. Those who were assigned to the SPACE for COPD group received an introduction to the SPACE for COPD manual and 2 telephone calls at 2 and 4 weeks. Those assigned to PR received 14, 2 hour sessions of education and exercise over 7 weeks. The primary outcome was the Chronic Respiratory Questionnaire (CRQ) dyspnoea, with secondary measure of CRQ fatigue, emotion and mastery, Incremental Shuttle Walk Test (ISWT) and Endurance Shuttle Walk Test (ESWT). Measures were taken at

Abstract S24 Table 1.

	SPACE for COPD n=94			Pulmonary Rehabilitation n=84			Between group differences
	Baseline (SD)	7 weeks (SD)	Change (95% CI)	Baseline (SD)	7 weeks (SD)	Change (95% CI)	(95% CI)
Dyspnoea	2.58(0.93)	3.11 (1.23)	0.55(0.26 to 0.85)p=<0.001	2.42 (0.91)	3.38 (1.18)	0.87(0.61 to 1.13)p<0.001	-0.32 (-0.71 to 0.08)p=0.113
Fatigue	3.42 (1.19)	3.71 (1.22)	0.13(-0.14 to 0.39)p=0.339	3.36 (1.20)	4.09 (1.49)	0.54(0.27 to 0.82)p<0.001	-0.41 (-0.79 to 0.03)p=0.033
Emotion	4.41 (1.24)	4.56 (1.20)	0.005(-0.23 to 0.24)p=0.965	4.37 (1.24)	4.92 (1.03)	0.53(0.26 to 0.79)p<0.001	-0.52(-0.88 to 0.17)p=0.004
Mastery	4.50 (1.40)	4.78 (1.31)	0.15(-0.11 to 0.40)p=0.259	4.36 (1.30)	4.94 (1.19)	0.58(0.34 to 0.81)p<0.001	-0.43(-0.77 to 0.09)p=0.014
ISWT (metres)	260.24 (147.91)	277.86 (145.59)	17.62(3.26 to 31.98)P=0.17	268.61 (149.89)	310.13 (156.46)	41.52(23.91 to 59.12)	-23.90 (-46.33 to -1.47)p=0.038
ESWT (seconds)	231.42 (231.00)	444.33 (393.09)	212.91(139.49 to 286.34)P<0.001	189.14 (96.25)	534.85 (395.38)	345.70(260.20 to 431.20)p<0.001	-132.78 (-244.63 to -20.94)p=0.02