

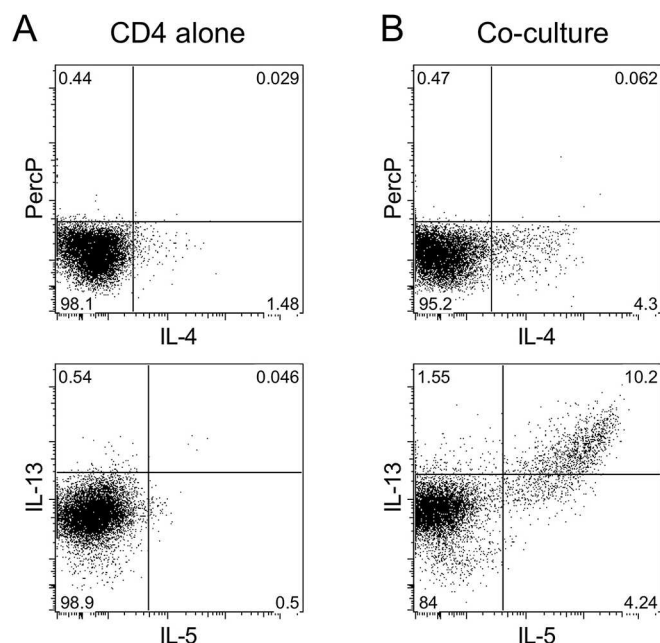
interleukin (IL)-33 and their production of type-2 cytokines (including IL-5, IL-9, IL-13). ILC2 have a variety of roles in lung inflammation and repair. Their interaction with innate immune cells has been shown, however their influence on the adaptive immune system remains unknown. Given the important 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<sup>+</sup>CD44<sup>lo</sup>) were sorted by FACS from ST2 knockout mice and were cultured alone (A) or with ILC2 (Lin<sup>+</sup>CD45<sup>+</sup>ICOS<sup>+</sup>ST2<sup>+</sup>) 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.

S22

## ANALYSIS OF THE LUNG MICROBIOME IN HUMAN ASTHMA USING WHOLE GENOME SHOT-GUN METAGENOMICS

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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 whippelii* 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.

S23

## MULTIDIMENSIONAL PHENOTYPES OF ASTHMA

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