THE EFFECTS OF INHALED CORTICOSTEROIDS ON HEALTHY AIRWAYS

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Background The effects of inhaled corticosteroids (ICS) on healthy airways are poorly described. Delineating their effects on gene expression in health, without the confounding changes generated by changes in disease-related genes, will enable more precise analysis of data in severe asthma.

Methods We performed a randomised open-label bronchoscopy study of high dose ICS therapy in 30 healthy adult volunteers randomised 2:1 to i) fluticasone propionate Accuhaler 500 mcg bd or ii) no treatment, for 4 weeks. Laboratory staff were blinded to allocation. Biopsies and brushings were analysed by immunohistochemistry, bulk RNA sequencing, DNA methylation area and metagenomics.

Results Blood eosinophil numbers increased after 4 weeks in the observation group compared to people using ICS. This was not related to atopic status. Blood neutrophils, FeNO or FEV1 did not differ between groups. There was a small significant between-group difference in change in lamina propria eosinophils but not for other immunological markers. ICS treatment caused upregulation of 72 genes in brushings, and 53 in biopsies, and down regulation of 82 genes in brushings and 416 genes in biopsies. The most upregulated genes were predominantly those involved in steroid metabolism, cellular proliferation, cellular metabolism and cytoskeletal changes. By contrast the most downregulated genes in both brushings and biopsies were key mediators of type 2 inflammation (FCER1A, CPA3, IL33, CLEC10A, SERPINB10 and CCR5) or of T cell mediated adaptive immunity (TARP, TRBC1, TRBC2, PTPN22, TRAC, CD2, CD8, HLA-DQB2, CD96, PTPN7). All other top 20 common downregulated genes were involved with innate or adaptive immunity, including Hobit, RANTES, Langerin and GFI1. Genest enrichment showed differential upregulation of 26 genes previously reported as induced by fluticasone in asthma. We observed minimal differences in the microbiome or DNA methylation.

Conclusions We defined genes altered directly by corticosteroid therapy without confounding by disease. These were predominantly downregulated genes. Strikingly even in health the most downregulated genes were canonical markers of type 2 inflammation. This implies that homeostasis in health involves tonic type 2 signalling in the airway mucosa, which is very sensitive to corticosteroids.

Please refer to page A285 for declarations of interest related to this abstract.

PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS OF RESIDUAL STEROID-RESPONSIVE INFLAMMATION IN MEPOLIZUMAB TREATED PATIENTS

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Background Mepolizumab is an anti-interleukin-5 monoclonal antibody for severe eosinophilic asthma (SEA). The additional
CIRCADIAN PATTERNS IN IMMUNE CELL TRAFFICKING

Aim Determine the transcriptomic and proteomic effects of prednisolone versus placebo on the airways and blood in patients with SEA treated with mepolizumab.

Methods MAPLE was a randomized, double-blind, placebo-controlled crossover trial of prednisolone at stable state in adults with SEA after mepolizumab (Yang F, JACI Pract 2022;10:2925–34.e12). Prednisolone had a minor effect on FEV₁ but not on symptoms. Sputum and blood samples were taken before and after high dose prednisolone and placebo in patients treated with mepolizumab. These underwent O-link expression analysis of 1536 proteins. A paired comparison of normalised protein expression for 1536 proteins in sputum and serum were compared in a linear mixed effects model, with Benjamini-Hochberg correction for multiple testing.

Results 21 participants had paired serum, and 14 had paired sputum, before and after both prednisolone and placebo. Prednisolone significantly downregulated 173 and 229 proteins and upregulated 63 and 140 proteins in sputum and serum respectively. Downregulated proteins in sputum included IL-4, IL-5, IL-13, chemokines, and signatures of mast cells, prostanoids, and alternatively activated macrophages. Upregulated proteins included FKBP5, typical of steroid treatment.

Conclusions Prednisolone significantly downregulated type-2 pathways unaffected by IL-5 inhibition in the sputum and blood proteome, and nasal transcriptome. These findings support the notion that the type-2 airway epithelium remains active in mepolizumab-treated patients. The relationship of these additional effects to longer term clinical outcome is unknown.

Please refer to page A286 for declarations of interest related to this abstract.

CIRCADIAN PATTERNS IN IMMUNE CELL TRAFFICKING IN CHRONIC ALLERGIC AIRWAYS DISEASE

Background Asthma is a chronic inflammatory disease, commonly featuring an eosinophilic allergic-type signature. Patients with asthma experience diurnal variation in symptoms, with notable worsening in lung function around 04:00, coinciding with peak sputum eosinophilia. A better understanding of the rhythmic inflammatory pathways underpinning asthma may identify novel therapeutic targets.

Experimental Design Female C57BL/6 mice were exposed intranasally to House Dust Mite (HDM) or Phosphate Buffered Saline (PBS) 3x per week for 5 weeks. Tissues were harvested at 6hr intervals over a 72hr period. Immune cell populations in lung, bronchoalveolar lavage fluid (BALF), spleen, and blood were characterised (flow cytometry) and BALF and serum cytokines quantified (bioplex). qPCR and Immunohistochemistry assessed expression of tight junction components found in the airway epithelium. T-helper 2 cells were further analysed to show varying expression of the immune modulatory transcription factor E4BP4 in BALF, lung tissue, and peripheral blood.

Results Immune cell populations in HDM-exposed mice showed time-of-day variations. Eosinophils and Th2 cells peaked at 07:00 in BALF and reached a nadir at 19:00. Similarly, serum IL-5 peaked at 07:00. Blood eosinophils however displayed a consistent daily peak at 13:00 (figure 1), in both HDM and PBS exposed mice. Diurnal variation in the expression of tight junction genes zo-1 and ocn was lost in HDM-exposed mice indicating airways may be leakier, especially at 07:00. T-helper 2 cells, exhibit a reversed pattern of expression of the E4BP4 protein in the lung tissue compared to in the blood and BALF, perhaps driving the cells towards a more proinflammatory phenotype.

Perspectives Our murine model of chronic allergic inflammation demonstrates time of day variation in the cellular milieu of the lung. During the rest phase (when asthma symptoms are typically worse) levels of the eosinophil chemoattractant IL5, lung eosinophils and barrier permeability are heightened. Ongoing work will address mechanisms facilitating rhythmic eosinophil accumulation and clock gene function in T-helper 2 cells when present in the inflamed lung.

Please refer to page A286 for declarations of interest related to this abstract.

COMPUTED CARDIOPULMONOGRAPHY: AN INNOVATIVE ASSESSMENT OF LUNG FUNCTION BEFORE AND AFTER STARTING BIOLOGIC THERAPY FOR TH-2 HIGH ASTHMA

Background Anti ILS/anti-IL5R biologics are highly effective in asthma by targeting eosinophilic inflammation and reducing exacerbations, but their effects on lung function are less clear. The aim of the study was to explore whether a novel lung function index, eCLnCL, provided by a new technique, computed cardiopulmonaryography (CCP), is modified following treatment with these biologic therapies in patients with severe type-2 high asthma. eCLnCL measures inhomogeneity (unevenness) in lung tissue inflation/deflation and is a sensitive index of small-airways disease.

Methods This was an observational study at a tertiary asthma clinic. Fifty-four patients with type-2 high asthma were evaluated at baseline and following their 4th biologic injection with an anti-ILS or anti-IL5R agent 3 or 4 months later, respectively. Assessments included CCP (as described in [1]) and standard spirometry, both pre- and post-bronchodilation with salbutamol, and measurements of blood eosinophil count (BEC), FeNO and ACQ5.

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