23 May 2018

EAACI-Allergopharma Research Award 2017, Final Report

I am very honored to be the recipient of the EAACI-Allergopharma Award 2017. This award is an important recognition of my work, in particular my recent publication 'MicroRNA-155 is a critical regulator of type 2 innate lymphoid cells and IL-33 signaling in experimental models of allergic airway inflammation' (Johansson et al. 2017 *J Allergy Clin Immunol*). I am truly grateful for the support and encouragement to my research from EAACI and Allergopharma.

The final report of my ongoing project 'MicroRNA Regulation in Asthma' is attached to this letter.

Sincerely,

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MicroRNA Regulation in Asthma

The project 'MicroRNA Regulation in Asthma' was carried out during 2017 as part of my doctoral studies at Krefting Research Centre at the University of Gothenburg in Sweden. I began my studies of immunological mechanisms in asthma and allergy at Krefting Research Centre in 2014, and I defended my PhD thesis entitled 'ILC2s and miRNA regulation in allergy and asthma' in May 2018.

Summary

The overall aim of the current project is to increase the understanding of antiviral defense mechanisms in airway macrophages that might contribute to virus-triggered exacerbations in asthma. MicroRNA (miRNA) expression in poly(I:C) (i.e. viral mimic) stimulated airway macrophages from asthmatic subjects and healthy controls were analyzed by microarray. With support of the EAACI-Allergopharma Award 2017 we have analyzed inflammatory mediators in bronchial lavage from asthmatic and healthy subjects, and in culture media from poly(I:C) stimulated airway macrophages. The results are currently being analyzed and will be linked to functional investigations of miRNA-gene target networks in asthmatic versus healthy cells.

Background

MicroRNAs (miRNAs) are small noncoding RNA molecules that mediate sequence-specific repression of target messenger RNAs (mRNAs), inhibiting gene expression at the post-transcriptional level (1). miRNAs shape cellular responses in health and disease by regulating fundamental cellular processes such as proliferation, differentiation, migration and apoptosis (2-4). A single miRNA may target multiple mRNAs and an individual mRNA may be directly regulated by several different miRNAs. This is highly context-dependent and varies with cell type, tissue or even cell status. Nevertheless, this complexity also represents the strength of miRNAs which often regulate networks of functionally related gene transcripts involved in common biological pathways. It means that altered expression of a single miRNA may change the course of an inflammatory process and affect disease progression (5-7). This, of course, makes miRNAs highly attractive for therapeutic intervention, where the miRNAs themselves, or downstream gene products, might be targeted.

Alveolar macrophages are found in the airway lumen, in close proximity to the mucosal surface which typically makes them the first line of defense against inhaled particles of pathogens. The frequency of macrophages in asthmatic airways have been reported at comparable levels to

healthy controls (8), however, the function of alveolar macrophages has been suggested to be altered in asthma. For example, alveolar macrophages from children with poorly controlled asthma demonstrated decreased phagocytosis and increased apoptosis compared to cells from healthy donors (9). Furthermore, macrophages are important regulatory cells in airway inflammation and hyperreactivity in experimental models of asthma (10-14), and a link between lung macrophages and airway remodeling has been established in human asthma (15-16). Macrophages are also involved in immunological defense to respiratory viruses and this is an area of clinical importance in asthma as viral infections are the most common trigger of asthma exacerbations (17).

Objective

The aim of this study was to uncover antiviral pathways mediated by macrophages that contribute to asthma pathology. Identification of dysregulated miRNA expression might lead us to target genes that control pathological responses in asthma, therefore, we sought to determine whether miRNAs were differentially expressed in primary airway macrophages from asthmatic individuals compared to healthy controls.

Preliminary results

Study participants were recruited from the West Sweden Asthma Study cohort. Written informed consent was obtained from all subjects included and ethical approval was issued from the Gothenburg County Regional Ethical Committee. The cohort was initiated at Krefting Research Centre in 2008 as a large-scale population-based study focusing on asthma and allergic diseases in west Sweden. Detailed description of the study material is provided in (18-20). Briefly, a postal questionnaire was sent to 30,000 individuals living in the area, aged 16-75 years. Out of 18,087 responders, 2,006 randomly selected subjects and additional subjects reporting ever having asthma or physician-diagnosed asthma underwent clinical phenotyping. This included interviews, lung function tests (FEV_1 and FVC) before and after bronchodilation, test of methacholine responsiveness, skin prick test, F_{ENO} measurement and blood (serum and plasma) collection. 20 asthmatic subjects and 10 healthy controls were invited for re-phenotyping which included interviews, physiological tests and blood sampling as described above. All subjects were under the age of 75 years, non-smokers (including ex-smokers for >5 years, <10 pack years) with no autoimmune disease or cancer. Asthmatic subjects had physician-diagnosed asthma, defined by clinical history, reversibility (FEV_1>15%) or positive methacholine challenge. All asthmatics reported current ICS use. Allergic status was assessed by skin prick test. Age- and sex-matched healthy controls who did not report asthma symptoms

were recruited. They were non-reactive to methacholine or non-reversible. Asthmatic and healthy subjects fulfilling inclusion criteria were invited to undergo bronchoscopy with bronchial lavage within <4 weeks. The day of the bronchoscopy additional samples were collected to screen for signs of current inflammation including C-reactive protein (CRP) levels and leukocyte numbers (differential cell count) in peripheral blood and respiratory virus infection by nasal swab.

Macrophages in bronchial lavage were enriched by adherence to cell culture plates. Suspension cells were washed away and the macrophages were rested for 48 h before poly(I:C) stimulation to mimic a viral infection. miRNA expression in poly(I:C) stimulated macrophages and unstimulated controls were analyzed by microarray. A majority of the detected miRNAs were present in macrophages isolated from both asthmatic and healthy airways, and a limited number of miRNAs were uniquely expressed by either of the two groups. miRNA expression profiles were significantly altered upon poly(I:C) stimulation, and miRNA expression levels are currently being validated by qPCR. Furthermore, cell-free culture supernatants were analyzed by multiplex immunoassay (Olink Proteomics, Uppsala, Sweden). Our preliminary data demonstrate that poly(I:C)-stimulation resulted in release of pro-inflammatory mediators by macrophage cultures. Importantly, macrophages from asthmatic subjects, but not healthy subjects, induced inflammatory mediators that have previously been reported at elevated levels in asthma. In our continued analysis we will connect miRNA expression profiles to inflammatory mediators to discover antiviral pathways that might contribute to asthma immunopathology.

Literature cited

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