Medical University of Graz

Clock genes as a therapeutic target for asthma

Summary

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Supervisor:	PD Dr. Eva Sturm
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 10, 2020 00:00 and March 30, 2020 23:59 (Europe/Zurich)

Description

Background. Asthma is an inflammatory disease with a strong circadian signature. Symptoms of asthma are worse overnight and in the early hours of the morning. Measurements of peak expiratory flow rate and forced expiratory volume in 1 second, are lower in early morning compared to afternoon. Clock genes control the circadian rhythms within every cell in the body. The clock gene REV-ERB alpha (NR1D1) is known to gate between the 'core' clock and the immune system. Thus, it is likely that circadian biology plays a crucial role in the pathogenesis of asthma. Improved knowledge of the circadian nature of asthma phenotypes may lead to better treatments through chronotherapeutic drug delivery (taking medication at the most beneficial time of day).

Hypothesis and Objectives. Based on preliminary data we hypothesize that REV-ERB alpha plays a crucial role in the pathophysiology of allergic disorders by regulating eosinophil, neutrophil and macrophage function. In this project we aim to (i) investigate the signaling of REV-ERB alpha and other related clock genes in peripheral blood leukocytes from allergic and non-allergic asthmatic patients as compared to healthy controls. (ii) As clock gene expression and signaling can change upon cell activation infiltrated leukocytes in BAL fluid and nasal mucus will be evaluated. To prove the *in vivo* relevance of these approaches, (iii) two well-established murine *in vivo* models of CCL24-directed eosinophil migration into the airways and (iv) three different models of OVA-induced airway inflammation which reflect asthma phenotypes seen in humans: eosinophilic, neutrophilic and mixed-granulocytic airway inflammation will be used to confirm our hypothesis.

Methodology. The PhD student will learn how to isolate leukocytes, especially eosinophils and neutrophils, from peripheral blood and BAL fluid by negative magnetic selection. Clock gene signaling and expression will be quantified by real-time PCR, Western blot and fluorescence microscopy **(Year 1-2)**. Functional responses of eosinophils and neutrophils will be investigated in assays of shape change, integrin up-regulation and chemotaxis by flow cytometry **(Year 1-2)**. Moreover, the student will be trained in well-established experimental mouse models for eosinophil directed migration and allergic asthma in wild type and REV-ERB alpha knockout and transgenic mice **(Year 3-4)**. Cytokine profiles will be determined by multiplex ELISA. Lung tissue will be analyzed by immunohistochemistry and *in situ* hybridization. Lipid mediators will be determined by mass spectrometry. To further investigate the role of clock genes in lung biology differential gene expression will be evaluated by RNA sequencing **(Year 3-4)**. All *in vitro* and *in vivo* investigations will be performed at three different times of the day. To study clock gene expression and signaling endogenous and synthetic agonists and antagonists will be used.

Collaborations within the MUG. PV. Tomazic will collect nasal mucus samples from allergic patients. H. Olschewski will recruit asthmatic patients and collect blood, plasma and BAL samples.

Input from collaborations within the DK-Molin programme. The group of G. Kwapiszewska will train the student in isolation techniques for inflammatory cells from lung tissue and G. Kwapiszewska agreed to support the student with her expertise in experimental mouse models of lung diseases and lung pathology. A. Heinemann will support the student with his expertise in allergic and asthmatic diseases and collect blood and plasma samples from allergic and healthy donors.

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Input from international collaborating research groups where the PhD students could carry out parts of their projects abroad. D. Thomas (University of Frankfurt, Germany) will train the student in mass spectrometric analysis of lipid mediators in *in vivo* and *in vitro* samples.

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