Study of airway inflammation as a result of external triggers inducing epithelial cell damage in non-allergic asthma and exercise-induced bronchoconstriction

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In this thesis the impact of external triggers on airway inflammation was investigated, with a focus on adolescent athletes and individuals with asthma. The study explores the complex relationship between airway epithelium, immune cells and bronchoconstriction, particularly in the context of mast cell activation regarding the underlying mechanism. Additionally, it examines exercise-induced bronchoconstriction (EIB) and the influence of environmental factors on airway health in athletes.

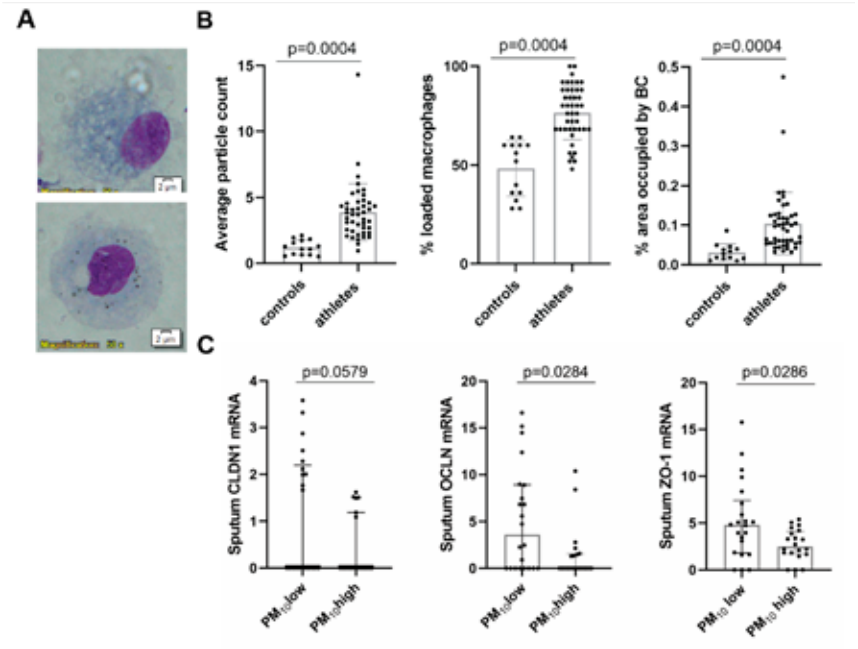
Human airways are continuously exposed to external triggers trough breathing, which can initiate epithelial damage. This may induce an inflammatory response, resulting in bronchoconstriction. It is known that in asthma, which is an heterogenous disease characterized by reversible airway obstruction, there is a complex interaction between airway epithelium and immune cells in the initiation and continuation of airway inflammation. As mast cells are located close to the airway epithelium, we hypothesize they are critical in mediating this response. Released mast cell mediators via both IgE-dependent and IgE-independent mast cell activation are able to induce bronchoconstriction. The role of the newly described Mas-related G-protein coupled receptor member X2 (MRGPRX2) in this cascade is not fully understood (1). Furthermore, the thesis addresses the occurrence of bronchoconstriction in otherwise healthy individuals, a phenomenon known as EIB. Athletes, in particular, face an elevated risk of EIB, with factors such as the intensity of sporting activities and external triggers like cold air in cross-country skiing or chlorine by-products in swimming contributing to its development (2). Even adolescent athletes embarking on their professional careers are susceptible to EIB, underscoring the need for better identification and management of this condition among them (3, 4).

The main objective of this thesis was to study airway inflammation as a result of external triggers inducing epithelial damage. We hypothesize that adolescent athletes, due to their heightened ventilation rates, are more vulnerable to external triggers, which might act as stressor to the airway barrier. The research is structured around three main objectives: first, studying atopy and EIB in intense adolescent athletes; second, delving deeper into the impact of external stimuli on the airways of elite adolescent athletes and asthmatics; and finally, examining the central role of mast cells in airway inflammation.

Atopy has been significantly associated with bronchial hyperreactivity and EIB in adult elite athletes (5). Therefore, a screening tool may help with the early identification of atopy and allergy symptom development, which may impact physical performances in adolescent athletes. An Allergy Questionnaire for Athletes (AQUA©) score of ≥ 6 and fractional exhaled nitric oxide (FeNO) levels of ≥ 15 ppb were identified as prediction tool for EIB in adolescent elite athletes (12-18 years) (6, 7). These results were confirmed in recreational athletes performing at least 12 hours of sport a week (12-18 years). These results showed the presence of atopy in approximately 40% of adolescent athletes in both cohorts, which is higher than in the general population. Furthermore, 14% of recreational athletes reported previous asthma diagnosis and 22% tested positive for EIB. Of these EIB+ athletes, 76% of athletes did not receive a prior asthma diagnosis, which is often used interchangeably in real life practice. These results indicate the need to better identify EIB in adolescent athletes. Investigating different factors linked to EIB, the highest sensitivity was found for AQUA© ≥ 6 and highest specificity was found for reporting wheeze during exercise. Furthermore, previous asthma diagnosis was associated with outdoor athletes, highlighting the impact of the environment during intense exercise. Serum levels of epithelial damage biomarkers were not able to differentiate EIB+ and EIB- athletes, but were associated to training type, training intensity and EIB severity.

Secondly, an in-depth exploration of the effect of external stimuli on the airways of elite adolescent athletes and asthmatics was performed. The effect of intense exercise and environmental exposure to air pollution

Figure : Adapted from Goossens et al. Thorax 2023 (8). (A) Illustration of images captured for analysis showing airway macrophages stained by diff-Quick with increasing black carbon load. (B) The average particle count per macrophage, percentage of loaded macrophages, and the percentage area occupied by black carbon for each participant was calculated by a blinded researcher. For each participant 25 macrophages were counted. (normality confirmed, unpaired t-test with Welch's correction). (C) Effect of PM10 on tight junction expression of claudin 1 (CLDN1), occludin (OCLN) and Zonula occludens (ZO-1). (Mann-Whitney)



on the airways of adolescent elite athletes was investigated. Indeed, RNA-Seq analysis of sputum transcriptome showed significantly differentially expressed genes in athletes compared with controls, which were related to inflammation and epithelial cell damage (8). In addition, sputum samples of athletes contained significantly more carbon loaded airway macrophages compared with controls (figure 1A,B), likely the result of their high ventilatory demands during exercise. In addition, significantly lower mRNA levels of OCLN and ZO-1 in athletes exposed to higher particulate matter ≤10µm (PM10) levels compared with athletes exposed to lower levels were observed (figure 1C). Remarkably, the airway response to eucapnic voluntary hyperpnoea (EVH) testing in athletes was associated to prior PM exposure, indicating that exposure to increased air pollution may induce short term increased airway hyperreactivity. Our preliminary RNA-Seq analysis between EIB+ and EIB- athletes suggested a role of epithelial damage, oxidative stress and (neuro)inflammation in EIB. A retrospective analysis was performed of environmental exposures of patients with asthma, including smoking and work-related exposures. Increased epithelial damage in asthmatic patients compared with healthy controls was demonstrated, suggesting that they might be more vulnerable for external triggers. We indeed found significant differences amongst sputum transcriptome of asthmatics exposed to cigarette smoke or work-related exposure to cleaning products compared with asthmatic patients without exposure. A role for the aryl hydrocarbon pathway (AhR) for airway inflammation in asthmatic patients exposed to irritants was suggested.

Lastly, the involvement of mast cells in non-IgE mediated airway inflammation was investigated. In this thesis, a pilot study was performed in asthmatic patients compared with healthy controls to characterize MRGPRX2 expressing mast cells in sputum samples. Sputum mast cells were increased in allergic asthmatic patients compared with controls. However, also increased mast cell activation was observed in non-allergic asthma. MRGPRX2 expression was not associated with allergic or non-allergic asthma phenotype. Furthermore, neuromediator Neurokinin A (NKA) correlated positively with the percentage of mast cells and negatively with FEV1/FVC. These results suggested a role for mast cells in neuro-immune reaction for both allergic and non-allergic asthma patients. To better investigate this role of the mast cell, a human mast cell differentiation protocol was optimized to obtain functional MRGPRX2 expressing mast cells. Stimulation of mast cells with substance P resulted in increased CD63 expression and the classical inhibitor ketotifen was able to inhibit this activation. The optimized in vitro model can be used to explore the role of mast cells in especially MRGPRX2 mediated activation and as screening tool for potential therapeutics.

In conclusion, this research underscores the increased vulnerability of intense adolescent athletes to environmental triggers and epithelial damage. It emphasizes the need for robust screening tools to monitor and identify athletes at risk of EIB. Moreover, the impact of external triggers on the airways extends to asthmatic individuals, where the involvement of non-IgE mediated mast cell activation is proposed. The development of research tools for investigating MRGPRX2-mediated activation holds promise for future studies and therapeutic advancements in this area.

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