The damaging and protective features of eosinophils in healthy individuals and patients with chronic inflammatory respiratory diseases

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Life is about pretty moments, enjoy this one - Hunkemöller
Chapter 1
General introduction &
Scope of this thesis
GENERAL INTRODUCTION

OBSTRUCTIVE AIRWAY DISEASES

Chronic obstructive airway diseases comprise asthma, chronic obstructive pulmonary disease (COPD) and non-cystic fibrosis bronchiectasis and are chronic inflammatory diseases characterized by airflow obstruction and subsequent airflow limitation.

ASTHMA

Asthma is a heterogeneous disease affecting approximately 300 million people worldwide. Despite this heterogeneity, asthma is associated with airway hyperresponsiveness, chest tightness, cough, wheezing, dyspnea and as a key feature reversible airflow obstruction, which could become permanent as a result of airway remodeling (1, 2). Asthma is probably caused by an interplay between both genetic susceptibility and environmental exposures like pollution, allergens and virus infections (3). These environmental exposures are also the predominant triggers for acute worsening of asthma symptoms, called an exacerbation (4). Exacerbations are paralleled by increased airway inflammation and a decline in lung function, which severely impact the patient's quality of life and in severe asthma may even be life-threatening.

COPD AND BRONCHIECTASIS

With an equal incidence as asthma, COPD too is a heterogeneous disease, also caused by an interplay between genetic susceptibility and environmental exposures, be it different from those involved in asthma, i.e. tobacco smoke and household air pollution. Patients with COPD and bronchiectasis also have chronically inflamed airways and often experience exacerbations, paralleled by increased inflammation (5, 6). As in asthma, COPD is also characterized by airflow obstruction, however, in COPD this is not reversible and progressive (5, 7). Bronchiectasis is a highly heterogeneous disease, because it represents a final common pathway in different airway diseases. Bronchiectasis is caused by a pathologic dilation and mucosal thickening of the small and medium-sized bronchi (8, 9).
AIRWAY INFLAMMATION

The inflammatory profile in both asthma and COPD is well-characterized, in contrast to that for bronchiectasis. In general, allergic asthma is represented by a Th2 profile (5, 10, 11), reflected by a relative abundance of eosinophils (>3% sputum eosinophils). The specific mediator of cytokines IL-4, IL-5 and IL-13 play an important role in the pathogenesis of allergic airway inflammation in asthma (12). IL-4 and IL-13 are key players in mediating an allergic IgE response, recruitment of eosinophils via IL-13-mediated upregulation of VCAM-1 and ICAM-1, and mucus production (13), while IL-5 is crucial in the recruitment, activation and survival of eosinophils (14). Whereas allergic asthma patients can be properly controlled by corticosteroids, this is not the case for non-allergic and often more severe and corticosteroid-unresponsive asthma. In sputum from these patients there is a relative abundance of neutrophils and Th17-related cytokines like IL-17A. Eosinophilic and neutrophilic inflammation is also manifest in stable COPD (15).

Eosinophils and neutrophils are inflammatory effector cells, having the capacity to execute harmful responses to eradicate pathogens, but also damage tissue. The studies in this thesis focus on the role of eosinophils in chronic obstructive airway diseases, particularly in asthma.

EOSINOPHILS AND ASTHMA

Eosinophils are granulocytic bi-lobed leukocytes and can traffic to mucosal tissues. Only a small number of eosinophils reside in the circulation under homeostatic circumstances (<300 or <400 cells/µl blood) (16). In their specific granules, eosinophils store preformed enzymatic and non-enzymatic cationic proteins i.e. major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO). These cationic proteins can be excreted selectively by activated eosinophils via degranulation and are toxic to cells and tissue (17). However, this excretion of cytotoxic proteins is beneficial in an anti-helminth response. Through the release of the toxic effector mediators MBP (18), ECP (19) and reactive oxygen species (ROS) eosinophils help in clearance of helminth infections (20). The same release of toxic mediators is involved in tumoricidal activities. Different studies showed the recruitment and degranulation of eosinophil at the tumour site and that the increased numbers of eosinophils leads to the inhibition of tumour growth (21).
In asthma and allergy, eosinophils are considered to have a predominant adverse role, but their exact contributions are far from clear. For a long time, eosinophils in asthma were assumed to be end-stage cytotoxic effector cells only as based on the release of granular contents (figure 1). Recently, also an alternative mode of eosinophil activation has been described, the expulsion of DNA that can capture micro-organisms and is referred to as EETosis, that could also explain the release of granular constituents (22). Whether this occurs and is relevant in asthma is unknown as yet.

**FIGURE 1.** Schematic overview of the role of eosinophils in a Th2 inflammatory response in asthma.
Whereas in the airway mucosa from healthy individuals there are no eosinophils present in any significant numbers, in many but not all asthma patients there are enhanced numbers of eosinophils present. Recently it was proposed that in an experimental asthma model there were tissue-resident eosinophils, which were claimed to have a role in homeostasis (23, 24). So far, however, the presence of these tissue-resident eosinophils in the airway mucosa from asthma patients has not been confirmed.

**VIRUS INFECTION**

Respiratory viruses are known to be major triggers for asthma exacerbations, both in adults and children (25). Respiratory viruses including Influenza, Respiratory syncytial virus (RSV) and Rhinoviruses (RV) are associated with increased asthma pathology for 80% in children and 40% in adults (26). These viruses infect predominantly airway epithelial cells and less so macrophages and induce the production of inflammatory cytokines that in asthma patients lead to exaggerated innate and adaptive immune responses that involve recruitment of eosinophils into the airways. The current concept is that the activated eosinophils degranulate, release cytotoxic proteins and could contribute to airway pathology during an exacerbation (25-27). Recent studies provided new insights in a novel and more protective role for eosinophils, as they have been shown to modulate immune responses and promote virus clearance. (28, 29). Thus, although eosinophils may play a central role in airway inflammation and pulmonary pathology, it is becoming more clear that eosinophils could have an anti-viral role as well (30, 31).

**THERAPY**

High sputum eosinophil counts are associated with the severity of asthma symptoms (32). Hence several newly developed asthma therapies are focused on the reduction of eosinophil numbers. Corticosteroids play an important role in the maintenance therapy of asthma, particularly by attenuating airway eosinophilia. Although steroids are considered to be the most effective anti-inflammatory therapy in asthma, some patients are unresponsive to corticosteroids (33, 34). Therefore, alternative therapies are developed to reduce both activation and number of eosinophils in an attempt to reduce asthma pathology. IL-5 has a crucial role in eosinophil development and therefore it has been targeted for the development of asthma medications. Different monoclonal antibodies (anti-IL-5 or anti-IL-5R antibodies like Reslizumab, Mepolizumab...
and Benralizumab, respectively) have been tested in clinical trials (35), (Figure 2.1). However, effectiveness is strictly correlated with eosinophilia and asthma severity. Another potential therapeutic approach that may affect eosinophil activation and degranulation, but not eosinophil numbers, can be the targeting of phosphodiesterase-4 (PDE4) signaling (36, 37), which is prominently expressed in eosinophils (36). PDE4 controls cellular cAMP levels by hydrolysis of cAMP into 5’AMP (figure 2.2), by this degradation of cAMP by which immune and inflammatory responses are amplified (38-40). PDE4 inhibitors have been shown effective both in vitro as well as in animal models for asthma and COPD (41, 42). Moreover, clinical studies that used PDE4 inhibitor Roflumilast showed anti-inflammatory effects on granulocytes and an improved lung function and reduction of symptoms in both COPD and asthma patients (43, 44).

DIAGNOSTIC MEASUREMENTS

Both eosinophil and neutrophil counts in sputum are recommended for tailoring corticosteroid dosing in asthma (45). However, the current method is based on sputum processing and cell counts which is labor intensive and a multiple day process. This method also does not account for cells dying during the whole process and also requires specific expertise that is not available in the majority of the clinics. Since both

**FIGURE 2.** Schematic overview of the targets of alternative therapeutics used in asthma. Monoclonal antibodies directed towards IL-5 cytokine or its receptor (1). Inhibition of PDE4 results in an accumulation of cAMP (2).
EPO and MPO are abundantly present in eosinophils and neutrophils respectively, and both enzymes can be detected by spectroscopy, it would be interesting to explore whether spectral analysis could substitute for relative eosinophil and neutrophil counts.

**SCOPE OF THIS THESIS**

Eosinophils are part of chronic airway inflammation in stable patients with obstructive airway disease, but are also recruited to the airways of these patients during a respiratory viral infection. So far, murine studies only have indicated that eosinophils may also regulate homeostatic processes and thereby orchestrate the restoration of steady state in airways. This thesis comprises studies on quantifying eosinophilic inflammation, assessing and modulating activities of eosinophils, and on an anti-viral and immune-modulating role for eosinophils both in vivo and in vitro, in mild and moderate asthma patients compared to healthy subjects, challenging the paradigm of eosinophils as detrimental and inflammatory cells and presenting eosinophils like a ‘double-edged sword’ of the immune system.

The aim of this thesis therefore encompasses:

1. Examine both in vivo and in vitro the interaction between eosinophils and respiratory viruses and investigate anti-viral properties, in men and mice, and in health and asthma
2. Examine the immune-modulating role of eosinophils by reducing their numbers by anti-IL-5 treatment in RV16 challenged mild to moderate asthma patients.
3. Investigate whether eosinophils contribute to oxidative stress in RV16 challenged mild to moderate asthma patients
4. Investigate the effects of PDE4 inhibitors on the cellular functions of both eosinophils and neutrophils from healthy and asthmatic subjects.
5. Examine the inflammatory and eosinophilic profile in bronchiectasis and exacerbations upon azithromycin or placebo treatment.
6. Development of a rapid assay for sputum granulocytes in asthma.
REFERENCES


