Airway inflammation in asthma : from concept to the clinic

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Chapter
8

Summary and general discussion

Summary

Airway inflammation is the key characteristic of asthma. In this thesis, we have investigated different aspects of this inflammatory process in patients with asthma. First, the pathogenesis of the disease was explored in a proof of concept study. Second, monitoring of airway inflammation was studied by examining markers of airway inflammation. Third, treatment of airway inflammation was investigated by studying improvements for asthma management. The main conclusions from these studies are summarized below.

Proof of concept

In **chapter 2**, we have shown that exogenous IL-5 is mainly effective in the circulation by enhancing the number of eosinophils to the circulation. There was no effect on the number of eosinophils in the lungs or on airway hyperresponsiveness. This suggests that IL-5 is able to promote the recruitment of eosinophils to the circulation.

Monitoring

The steroid-induced changes in airway hyperresponsiveness, sputum eosinophils and exhaled NO were compared in **chapter 3**. Treatment with inhaled steroids leads to significant improvements in airway hyperresponsiveness, reduced numbers of eosinophils in sputum, and decreased levels of exhaled nitric oxide in patients with asthma. The steroid-induced changes for each of the three different markers were not related. Therefore, the data suggest that these markers may provide different information when monitoring anti-inflammatory treatment in asthma.

Microvascular leakage is an important feature of inflammation. In **chapter 4**, an investigational model of "dual induction" was introduced to determine the level of microvascular leakage in patients with asthma. Using this model, it was possible to detect microvascular leakage by first inducing leakage with inhaled substance P and then measuring leakage in induced sputum. Alpha-2-macroglobulin appeared to be the most appropriate marker. This implies that this "dual induction" model can be applied when testing the antiexudative effect of newly developed drugs.

Chapter 5 demonstrated that the outcome of asthma, as determined by the annual decline in FEV_1, can be predicted by the bronchial CD8+ cell infiltrate. On the other hand, eosinophils in bronchial biopsies and the thickness of the sub-epithelial reticular layer were not associated with the decline in lung function. This suggests that inflammatory phenotypes in asthma may have prognostic relevance.

Management

In **chapter 6**, we have shown that PEF variability provides information about asthma severity in addition to symptoms and $ß_2$-agonist use. Patients who were classified as being in severity step 1 and 2 using the existing GINA criteria, but who had PEF-variability >10% had an almost 8 times higher risk for an increase in asthma severity 3 months later

compared to patients whose PEF-variability was ≤10% at baseline. Therefore, the current guidelines for the treatment of asthma can be improved by including PEF-variability in the assessment of asthma severity during treatment.

Treatment with anti-IgE, omalizumab, has recently been FDA approved for patients with moderate to severe persistent, IgE-mediated asthma that is sub-optimally controlled with inhaled steroids. We have demonstrated in **chapter 7** that PEF values were improved and that the response to inhaled allergen in asthma was diminished by anti-IgE. This was paralleled by a reduction in eosinophilic inflammation in bronchial mucosa and in induced sputum and a decline in bronchial IgE positive cell counts post-allergen. On the other hand, anti-IgE treatment did not improve airway hyperresponsiveness in these patients. This suggests that the clinical benefits of anti-IgE in asthma may be explained by a decrease in eosinophilic inflammation and IgE bearing cells. Furthermore, airway hyperresponsiveness appears to be independent of IgE.

General discussion

Proof of concept: is inflammation the right target?

Airway inflammation in asthma is complex in origin, regulation and outcome. There is still a lack of understanding of the mechanisms involved in asthma (1). Proof of concept studies may be useful to investigate the underlying pathogenesis of asthma. In this thesis we have performed a proof of concept study by questioning whether exogenous IL-5 leads to airway inflammation in asthma and whether the route of IL-5 production is crucial to its effects on the airways. IL-5 plays an important role in the mobilisation, differentiation and maturation of eosinophils (2). A causal relationship between the key pathological feature of asthma: eosinophils, and the key physiological characteristic: airway hyperresponsiveness, has been a long question of debate. The effects of inhaled steroids are suggestive for a causal relationship, since they both improve eosinophilia and airway hyperresponiveness in patients with asthma. On the other hand, the steroid-induced changes between sputum eosinophils and airway hyperresponsiveness were not related (Chapter 3). Some animal models, investigating the effect of IL-5 on eosinophilia, have shown the subsequent development of airway hyperresponsiveness, whereas others have not (3-6). In Chapter 2, we have demonstrated that intravenous administration of IL-5 to patients with asthma leads to an increased number of eosinophils in blood, but not in sputum. However, airway hyperresponsiveness was not affected. On the other hand, Shi *et al* did show an increase in airway hyperresponsiveness following IL-5 administration (7). This discrepancy may be related to differences in racial susceptibility to the effects of IL-5, since these studies were performed in ethnically different populations. The development of a monoclonal antibody against IL-5 further challenged the hypothesis that the eosinophil is the central effector cell in asthma (8). Anti-IL-5 treatment in patients with asthma abolishes eosinophils in blood and sputum, but this fall in eosinophils was not accompanied by changes in airway hyperresponsiveness or response to inhaled allergen (9). On the other hand, Flood-Page

and co-authors have demonstrated that anti-IL-5 treatment only partially depletes the numbers of eosinophils in bronchial biopsies of patients with asthma (10). Interestingly, anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane (11). The finding that the response to inhaled allergen is unaffected by anti-IL5 makes its unlikely that anti-IL5 treatment will be beneficial to control asthma. The drugs that are effective in asthma control (inhaled steroids, cromolyn, theophylline, leukotriene antagonists, and anti-IgE) all inhibit the late response to allergen. Long-acting $ß_2$-agonists also appeared to inhibit the late response by reducing airway inflammation (12). However, a study showed that long-acting $ß_2$-agonists modify allergen-induced airway responses through functional antagonism rather than the inhibition of inflammatory cell infiltration (13).

But, is it also true for airway hyperresponsiveness, that all effective asthma drugs improve airway hyperresponsiveness? Indeed, treatment with inhaled steroids, cromolyn, theophylline and leukotriene antagonists reduce airway hyperresponsiveness in asthma. Anti-IgE treatment, however, apparently has no effect on airway hyperresponsiveness after short-term treatment (Chapter 7) (14). Therefore, it seems that eosinophils and airway hyperresponsiveness are not causally related. Nevertheless, the clinical beneficial effect of anti-IgE treatment has been demonstrated in large phase 3 trials, involving both pediatric and adult patients with moderate to severe asthma (15-17). Based on these data, anti-IgE has recently been approved by the Food and Drug Administration in the US for patients with moderate to severe persistent, IgE-mediated asthma that is sub-optimally controlled with inhaled steroids. Furthermore, we have shown that in patients with mild persistent asthma treatment with anti-IgE for 12 weeks significantly improves morning and evening PEF and reduces early and late allergen response (Chapter 7). Thus, the question remains whether drugs have to reduce airway hyperresponsiveness in order to be beneficial for asthma treatment. Therefore, the issue arises which outcome parameters should prevail in proof of concept studies: the cellular and pathological outcome, or the functional endpoints?

What is the implication of the persistent airway hyperresponsiveness under anti-IgE treatment? IgE+ cells in the bronchial mucosa are significantly reduced following anti-IgE treatment (Chapter 7). Apparently, airway hyperresponsiveness in patients with asthma is independent of IgE. The clinical consequences of airway hyperresponsiveness are reflected in an increased variation in airway caliber both within and between days (PEF variability) (18). Indeed, PEF variability in our study did not change either. However, this may be explained by the low level of PEF variability in our patient group (Chapter 7). In patients with more severe asthma an effect of anti-IgE on PEF variability has not been published.

The pathological mechanisms responsible for airway hyperreponsiveness may be related to the altered behaviour of airway smooth muscle (10). Changes in the organization of contractile filaments or in the plasticity of smooth muscle may underlie the persistence of airway hyperresponsiveness (20). It could be argued that inhaled steroids are not only

anti-inflammatory, but also change the functional properties of airway smooth muscle, whereas anti-IgE treatment does not (21). Remarkably, it has recently been suggested in a pilot study, that anti-TNFα treatment reduces airway hyperresponsiveness and increases FEV_1, but does not affect inflammation (22). This may suggest that inflammation per se, is not the right target for asthma therapy. Nevertheless, inflammation in the airway wall may enhance airway narrowing during smooth muscle contraction and thereby lead to airway hyperresponsiveness (23). Interestingly, the number of mast cells in the smooth muscle of patients with asthma is inversely correlated with the PC_{20} methacholine in the subjects with asthma (24). Following anti-IgE, the number of mast cells in the lamina propria was not decreased (Chapter 7). Although, we have not analyzed the number of mast cells in the airway smooth muscle, this might provide an explanation for the unchanged airway hyperresponsiveness following anti-IgE treatment.

Monitoring inflammation: there is more than eosinophils

The current GINA guidelines recommend that lung function and symptoms are measured in order to adjust (anti-inflammatory) treatment and thereby maintain asthma control (1). It is an interesting hypothesis that more direct monitoring airway inflammation will lead to improved asthma control. Markers for monitoring airway inflammation were investigated in this thesis in three different ways. First, inhaled steroids improved airway hyperresponsiveness, sputum eosinophils and exhaled nitric oxide; however these changes were not interrelated. Therefore, these markers may provide complementary information when monitoring anti-inflammatory treatment in asthma (Chapter 3). Second, anti-exudative effect of treatments can be determined via the assessment of microvascular leakage in induced sputum following inhalation of substance P (Chapter 4). Third, CD8 cells in bronchial biopsies predicted lung function decline and thus demonstrated the prognostic value of inflammation in asthma (Chapter 5). Therefore, inflammation in asthma does not only consist of eosinophilic inflammation.

Which criteria can be identified to determine the usefulness of markers for monitoring inflammation in asthma? First, it is important to distinguish between markers for short- and long-term outcome of asthma. At this moment most research is focused on the short-term outcome of the disease. However, the inflammatory process within the airway may have different effects on short- and long-term outcome of the disease. Indeed, eosinophils have been shown to predict asthma exacerbations in a study with a follow-up period of 8 weeks (25). On the other hand, we could not observe the prognostic value of eosinophils in our follow-up study of 7½ years (Chapter 5). Therefore, the prognostic value of inflammatory markers may be different for short- and long-term follow-up. Markers for short-term outcome of asthma have to be safe, non-invasive, reproducible, accurate and easy to perform, since they have to be measured more often in the same patient. Furthermore, these markers should be responsive to the effects of (or to changes in) treatment, exposure or avoidance to allergens. This means that they should mirror changes in the degree of inflammation. Next, markers may be selected on their ability to discriminate between different diseases and thereby be of use for the diagnosis

of asthma. For example, the negative predictive value of airway hyperresponsiveness for asthma is very high (26). The diagnostic accuracy appears to increase if sputum eosinophils and levels of exhaled NO are used in comparison with conventional approaches as recommended in the guidelines (27). Alternatively, a marker should be able to reliably distinguish between different disease severities. Whether a marker meets this criterion is often tested by investigating the correlations with other measures of asthma severity (symptoms and lung function). However, the lack of such a correlation could also imply that this marker is reflecting a different component of the disease. We also failed to demonstrate a correlation between steroid-induced changes in airway hyperresponsiveness, sputum eosinophils and levels of exhaled NO (Chapter 3). This could imply that the first improvements induced by inhaled steroid for the different markers are "out of phase". On the other hand, it could mean that these three markers represent different features of asthma.

Measuring airway hyperresponsiveness has demonstrated its usefulness in asthma management (28). Although it is safe and non-invasive, it may not be easy to perform in a non-specialized setting. Nevertheless, measures of airway hyperresponsiveness may provide additional and useful information, which is probably not always picked-up by other markers of inflammation (29). Sputum eosinophils have also been effectively used to guide anti-inflammatory treatment (30). Again, specialized personnel time is needed for the induction and processing of sputum. Therefore, cost-effectiveness analyses are needed to investigate the repetitive use of these markers in regular patient care, outside a research setting. In Chapter 4, we have demonstrated that induced sputum can be used to assess microvascular leakage. Neurogenic inflammation, which may result in microvascular leakage, is an important component of the pathology in asthma (31). Monitoring this feature of the inflammatory process might also lead to better asthma control and should, therefore, be further explored. Improved asthma management based on monitoring the levels of exhaled NO has been used in adults and children (32;33). The measurement of exhaled NO is safe and non-invasive, but in contrast with AHR and sputum eosinophils, it is easy to perform. However, the equipment needed to measure exhaled NO is still very expensive. In the future, the measurement of exhaled NO might be used in regular patient care. On the other hand, it may be questioned whether these asthma management studies are sufficient proof that exhaled NO is a appropriate marker for adjusting treatment. Indeed, the levels of exhaled NO appeared not to predict loss of asthma control (25). Interestingly, the same study showed that changes in sputum eosinophils were prognostic for loss of control (25). The study by Leuppi and co-authors confirmed that both AHR and sputum eosinophils, but not exhaled NO, are predictive for asthma exacerbations (34).

Assessing airway inflammation for markers of long-term outcome is important for investigating the underlying mechanisms of the disease and for following the progression and resolution of the disease. Consequently, the criteria "non-invasive" and "easy to perform" may be less important, since these measures will not be performed frequently. The prognostic significance of airway inflammation for the long-term outcome of asthma

is still unclear. The lack of scientific evidence is probably due to the long duration needed to investigate markers for longitudinal follow-up. To overcome this problem, several cross-sectional studies have been performed. These cross-sectional studies have shown associations between eosinophils and persistent airflow limitation (35;36). Furthermore, others have demonstrated that the thickness of the sub-epithelial reticular layer was inversely associated with the level of lung function in asthma (37;38). Still, it is not known whether these cross-sectional associations hold after long-term follow-up. Indeed, we have found that the cross-sectional associations with eosinophils and sub-epithelial reticular layer thickness are not established in a longitudinal follow-up study (Chapter 5). It is not unexpected that cross-sectional and follow-up studies have different results, since cross-sectional studies do not include changes over time. Thus, longitudinal follow-up studies are required to examine the usefulness of markers for monitoring inflammation of long-term asthma outcome.

Management: how to distinguish asthma severity from asthma control?
Asthma is a heterogeneous disease. Patients who participate in clinical trials have to be classified according to the severity of their disease for enrolment. Traditionally, asthma severity is defined by the clinical features that are present in the absence of therapy. This approach has also been used in the current GINA guidelines (Table 5-6) (1). Under appropriate treatment these clinical features should be absent, otherwise there is lack of control. To be able to identify patients who are at increased risk for exacerbation, there is a growing need for a distinction between asthma severity and asthma control (39-41).

What is asthma severity? Asthma severity is meant to grade the underlying disease state. In the current asthma guidelines, classification of asthma severity is assessed by the clinical features that are present before treatment (1). These clinical features would include symptoms and lung function. However, these clinical features are modified by therapy. Therefore, treatment level should be taken into account relating asthma severity to clinical symptoms (39). Asthma severity may vary from time to time in a single patient, however, changes in asthma severity occur only relatively slowly over time.

What is asthma control? Asthma control, on the contrary, is meant to grade the current expression of the disease as a result of treatment intervention. It is based on the goals of optimal treatment as described in the asthma guidelines. These goals include the absence of symptoms. On the other hand, minimal symptoms are allowed if they do not (or only minimally) require rescue medication. Furthermore, lung function should be normal or at least near the patient's best. Asthma control will also mean control of exacerbations. However, when defining disease control, inflammation should also be taken in to account. Moreover, chronic control of asthma would indicate the prevention of loss of lung function.
Control can be achieved by patient education, environmental control and adequate treatment. There are several reasons why patients with asthma may have poorly-controlled disease. The most important reason is failure to adhere to treatment recommendations. Indeed, poor compliance to asthma medication has been repeatedly

reported (42). Second, untreated non-asthmatic conditions (gastroesophageal reflux, rhino sinusitis, psychopathology co-morbidity) associated with asthma may lead to poor asthma control. Finally, very severe asthma could also lead to uncontrolled asthma, but only if the two reasons mentioned above are excluded (39).

Although measures to assess control and severity of asthma overlap, there should be emphasis on the distinction between asthma control and asthma severity. The common perception that well-controlled asthma is synonymous with mild asthma and that poorly-controlled asthma is synonymous with severe asthma is wrong. Asthma severity should be defined by the minimum medication required to achieve asthma control (39). PEF variability may be a measure that can be used to assess and improve asthma control. Indeed, we have shown that PEF variability provides information in addition to symptoms and $ß_2$-agonist use and may therefore be valuable to adjust therapy in order to prevent loss of asthma control (Chapter 6). The current GINA guidelines use PEF variability only for the initial assessment before treatment. For the ongoing assessment of asthma control during treatment, PEF variability is not included in the guidelines (1). Asthma management studies using airway hyperresponsiveness (28), sputum eosinophils (30) or exhaled NO (33) as a marker to adjust treatment have demonstrated that the current guidelines are not optimal and can be improved. In addition, a treatment algorithm, which includes the reduction of PEF variability, might also improve the asthma management guidelines. Furthermore, the use of a composite measure to determine asthma control has been proposed (43;44). In the current GINA guidelines the presence of one of the features of a severity step is sufficient to place a patient in that category (1). Finally, it has been suggested that the patient perception should be taken into account (45). This would imply the inclusion of patient-centred outcomes in the asthma management guidelines.

Directions for future research

The studies described in this thesis have gained more insight into airway inflammation in patients with asthma. Despite the growing knowledge about the concept, monitoring and management of asthma, many issues remain to be explored. Interesting questions for futures studies may include:

- Which parameters should prevail in proof-of-concept studies?
- What is the clinical implication of the persistent airway hyperresponsiveness under anti-IgE treatment?
- Can measurement of microvascular leakage be used to monitor airway inflammation and thereby improve asthma control?
- Is it effective to adjust asthma treatment based on the levels of PEF- variability?
- What is the cost-effectiveness of non-invasive markers of airway inflammation to monitor asthma treatment?
- Which markers are useful for monitoring inflammation of long-term asthma outcome?
- Is it possible to improve the current GINA guidelines by including non-invasive markers of airway inflammation or by using a composite outcome?

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