Allergen induced changes in bronchial hyperresponsiveness. A clinical and immunohistological study

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Chapter 12

SUMMARY AND GENERAL DISCUSSION

12.1. Summary

Asthma is characterised by episodic, variable airflow-obstruction and increased responsiveness of the airways to a variety of stimuli. Moreover, asthma is not a single disease entity but should be considered as a group of disorders which have in common the clinical manifestations of variable airways obstruction and hyperresponsiveness. The list of agents that can provoke bronchoconstriction, sometimes called "triggers" of asthma, is long and includes not only the antigens but also a wide variety of materials that appear unlikely to act through an immunological mechanism. These agents are often called "non-specific" stimuli and include many agents and/or events. The ability of these stimuli to provoke bronchoconstriction is a function of the responsiveness of the airway.

With our increased knowledge of the disease, during the past decades, more complex models of asthma have been constructed in which two areas currently seem to be of prime importance: bronchial hyperresponsiveness (BHR) and inflammation of the airways.

However, it is, perhaps, too simple to expect that there would be a straightforward association between inflammation and BHR, as both of these terms encompass a range of heterogeneous conditions. If inflammation in general does not cause BHR, it is of interest to know the type of inflammation that may do so, or under what particular circumstances.

BHR increases upon natural exposure to airborne allergens or occupational asthma inducers. Conversely, removal from environmental exposure often results in a decreased BHR. An enhanced BHR following the late asthmatic response (LAR) induced by allergen provocation in sensitized human subjects has been observed. Interest is being shown in changes in BHR which precede the late asthmatic response. Observations suggest that the tissue damage, which is supposed to cause the increase in BHR, may occur before the LAR is clinically evident.

It is still uncertain what distinguishes late asthmatic responders from nonlate responders. Such a distinction is of importance because the degree of the LAR correlates with the severity of asthma symptoms. The severity of the pre-existing BHR and the allergen-induced increase in BHR may predict whether patients develop a LAR. This will support the hypothesis of a

positive feedback loop, in which patients who are more hyperresponsive develop a LAR after allergen exposure, which in turn induces an increase in BHR.

Histological studies almost invariably show that asthma is accompanied by inflammatory changes in the walls of the airways.

It is suggested that the allergen-induced increase in BHR and the development of an early asthmatic reaction (EAR) and LAR may both depend on inflammatory changes. As a consequence, such mechanisms may explain the clinical usefulness of anti-inflammatory drugs against EARs, LARs and BHR.

Therefore the model of allergen provocation may provide clinical information, but also information about the dynamics of allergen induced changes in the bronchial wall. Trying to understand this model, it may give some answers as to why and how inflammation occurs in allergic asthmatics and may be related to the clinical picture of asthma.

This thesis deals with the effects of allergen provocation on bronchial hyperresponsiveness. Clinical and immunohistological aspects have been studied and will be summarised. To allow for critical evaluation of the results an overview is presented of the anatomy, morphology, and histopathology of the bronchial tree and the different aspects of bronchial hyperresponsiveness.

Chapter 1 deals with the anatomy and morphology of the bronchial tree.

Chapter 2 describes the pathology of the airways in asthmatics and several aspects of bronchial biopsies and bronchoalveolar lavage. **Chapter 3** gives an overview of bronchial hyperresponsiveness and deals with diagnosis, clinical aspects, agents, allergen-induced changes in bronchial hyperresponsiveness and mechanisms. Finally, in **Chapter 4** the aims of the studies are delineated.

Chapter 5 describes the effect of allergen provocation on bronchial hyperresponsiveness, measured with methacholine and adenosine 5'-monophosphate (AMP). The allergen induced changes in BHR are studied at 3 h and 24 h after allergen challenge, when the FEV_1 is returned to prechallenge values. The magnitude of the decrease in PC_{20} methacholine at 3 h correlates with the severity of the late asthmatic reaction. A significant decrease is observed in the PC_{20} methacholine and PC_{20} AMP at 3 h. In contrast to methacholine, no significant decrease can be demonstrated with AMP for the PC_{20} at 24 h.

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In **Chapter 6** the results of Chapter 5 are further analysed to see how Nedocromil sodium, an anti-inflammatory compound, interacts with the early asthmatic reaction (EAR), late asthmatic reaction (LAR) and allergen-induced increase in BHR, measured with methacholine. Nedocromil sodium inhibits the increase in BHR at 3 h and 24 h, diminishes the severity of the EAR and inhibits the LAR.

Chapter 7 further analyses the effect of Nedocromil sodium, however instead of looking after its effects on BHR, measured with methacholine, now AMP is used to measure the allergen-induced increase in BHR. Nedocromil sodium given before allergen challenge prevents the increased responsiveness to AMP at 3 h, but causes a decrease in PC_{20} AMP at 24 h. Desensitisation of the adenosine receptor during the LAR, that is prevented by Nedocromil sodium, may explain the increased responsiveness at 24 h.

Furthermore this chapter briefly deals with the allergen-induced increase in BHR, measured at a time point when allergen-induced bronchoconstriction is still present and the supposed beneficial effect of the cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced increase in BHR. The effect of the studied compound may be a consequence of the reduction of airway calibre and not of allergen-induced increase in BHR.

Chapter 8 deals with potential cellular differences in the lung on a bronchial and broncho-alveolar level between single early asthmatic responders and dual asthmatic responders. Dual responders have a significant higher number of eosinophils and activated (EG2) eosinophils in their bronchial lavage compared to the single early responders. No differences are observed in the number of $CD4^+$, $CD8^+$ and HLA-DR positive cells. The observed differences are mainly found in the bronchial lavage and not in the broncho-alveolar lavage.

Looking after the clinical effects of allergen challenge on BHR in chapters 5,6, and 7, in **Chapter 9** the effects of allergen challenge on inflammatory cells in the airways are studied before, 3 h and 24 h after house dust mite challenge, performing bronchial and broncho-alveolar lavage, and using differential cell counting and immunohistochemistry. Clear differences are seen between bronchial and bronchoalveolar lavage. Activated (EG2) eosinophils are significantly increased at 3 h and 24 h after challenge both in single early and dual asthmatic responders, compared with prechallenge values. The number of activated (EG2) eosinophils are significantly higher at 3 h and at 24 h in patients who clinically develop a late

asthmatic reaction. No significant changes are observed in the number of $CD3^+$, $CD4^+$, and $CD8^+$ cells 3 and 24 h after the challenge.

Chapter 10 describes the effects of allergen challenge on eosinophilic activity at 3 h and 24 h after challenge, measured in the bronchial submucosa, bronchial lavage and bronchoalveolar lavage in dual asthmatic responders. The numbers of activated (EG2) eosinophils are significantly increased both at 3 h and 24 h in the submucosa and bronchial lavage. A significant negative correlation is found between the number of activated (EG2) eosinophils in the submucosa and in the BL 24 h after the allergen challenge. At 24 h the amount of eosinophilic cationic protein (ECP) is increased in the bronchial lavage. A significant correlation is observed between the amount of ECP at 3 h and the log PD_{20} house dust mite.

In **Chapter 11** the changes in the number of activated (EG2) eosinophils and T-lymphocytes in the bronchial submucosa are described 3 h and 24 h after allergen challenge. Furthermore the interaction between these cells is discussed. After challenge, a significant increase in the number of $CD8^+$ T-lymphocytes and activated (EG2) eosinophils is observed at 3 h and 24 h after challenge in the bronchial submucosa. Strong positive correlations are found between activated (EG2) eosinophils before the challenge and $CD3^+$ T-lymphocytes at 24 h after the challenge, and between activated (EG2) eosinophils before the challenge and $CD4^+$ T-lymphocytes at 24 h after challenge. The change in $CD8^+$ T-lymphocytes between before and 24 h after the challenge correlates inversely with the severity of the late asthmatic reaction.

12.2. Conclusions

1. Allergen-induced increase in bronchial hyperresponsiveness is present between the early and late asthmatic reaction, as measured with a direct and an indirect stimulus. The pre-existing BHR and increased BHR at 3 h is related to the development of the late asthmatic reaction after allergen challenge.

2. The differences found in the increase in BHR between the direct stimulus methacholine and the indirect stimulus AMP support the view that direct and indirect mechanisms are involved in the allergen-induced increase in BHR.

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3. The beneficial effect of Nedocromil sodium on the allergen-induced changes in BHR indicates that the allergen-induced changes in BHR are mainly the result of allergen-induced inflammatory changes in the bronchial wall.

4. The number of activated (EG2) eosinophils in the bronchial lavage discriminates between single early and dual asthmatic responders.

5. Activated (EG2) eosinophils in the bronchial lavage are associated with the allergen-induced increase in BHR at 3 and 24 hours and the tendency to develop a late asthmatic response.

6. Allergen challenge causes an increase in activated (EG2) eosinophils before and after the late asthmatic reaction. Activated (EG2) eosinophils show a shift from the submucosa to the epithelial lining, as concluded from the biopsies and lavages.

7. Activated (EG2) eosinophils are not only effector cells, but are also pro-inflammatory cells playing an active role in the recruitment of other inflammatory cells in relation to allergen challenge. $CD8^+$ T-lymphocytes play a role in diminishing the late asthmatic reaction after allergen challenge in allergic asthmatics.

12.3. General discussion

As described, the aim of this study was to investigate the nature and development of the allergen-induced inflammatory responses in allergic asthmatic patients between the early and late and after the late asthmatic reaction. Moreover, we tried to define a causal relation between these inflammatory events and the allergen-induced changes in bronchial hyperresponsiveness and its relation to the late asthmatic response.
Having summarised the main results of the studies it is necessary to give these results a more general perspective by discussing their possible contribution to the understanding of the mechanisms of allergen-induced changes in bronchial hyperresponsiveness.

Bronchoalveolar lavage has increased our knowledge about the inflammatory events taking place in the lungs of asthmatics. Indeed in addition to clinical observations it has provided clues for considering inflammation as the basis of the disease. It was hoped that any cellular pattern observed would mirror the same processes occurring within the lung parenchyma. However, this proved not to be the case[1,2]. Bronchoalveolar lavage provides information about cells present at the epithelial surface of the lung. The rapid exchange of instilled fluid with extra cellular fluid in the lung complicates the measurements of soluble mediators of inflammation recovered by lavage.

Attention is focused now on biopsy findings in asthma and when appropriate, supplemented with lavage. Before discussing our results it is important to make some remarks concerning the procedure it self. It is suggested that fiberoptic bronchoscopy should only be undertaken in subjects with mild asthma, whose FEV_1 is >70% predicted and PC_{20} histamine or methacholine is >0.1 mg/ml when measured by the standard procedures of Cockcroft[3].

Because of this we have had long discussions whether it is ethically allowed and safe from a medical point of view using fiberoptic bronchoscopy, to perform lavage and taking biopsies on three separate occasions, before and after allergen challenge, knowing that the patient is very hyperreactive and may have discomfort.

In all the procedures we have not experienced any short- or long-term problems, but it should be stressed that the procedure must be explained to the patient, and must be carried out by experienced staff in a specialised unit designed for studies of this sort and not part of a routine clinical service.

Although it is suggested that the division of lavage specimens into wash and BAL does not add further information with regard to the distinction between asthmatics and nonasthmatics[4], it provides clear information in distinguishing between patients with only an early asthmatic reaction and dual responders. In addition, comparing bronchial lavage findings with submucosal findings, extra information is obtained about the dynamics of cell movement from the submucosa to the epithelial lining.

What does the lavage information add to our knowledge about single and dual responders? At first, single responders show signs of inflammation in the lavage, but less pronounced compared to dual responders.

Second, changes in the number of inflammatory cells do occur after allergen provocation, again less pronounced compared to dual responders. These findings indicate that the difference between single and dual responders is not a qualitative difference but a quantitative one. Down-regulation may take place in patients with asthma, but is probably insufficient.

We have the impression from the biopsies taken that patients with only a single early reaction show a larger increase in the number of $CD8^+$ T-lymphocytes 3 h after challenge than the dual responders. This impression is supported by the finding of a negative correlation between the severity of the late asthmatic response and the increase in the number of $CD8^+$ cells in the dual responders. It may thus suggest that down-regulation in single responders is better organised than in dual responders.

Our results partly endorse the opinion that lavage findings do not exactly mirror events taking place in the submucosa. However, this seems to be restricted to T-lymphocytes and not to eosinophils.

The evolving recognition of the capacity of eosinophils to engage in other types of cellular responses suggests that eosinophils can collaborate with lymphocytes and other immunologic and mesenchymal cells in various ways that are pertinent to health and disease. Eosinophils can express cell-surface proteins that may enable them to interact with lymphocytes. The ability of eosinophils, after *in vitro* induction of HLA-DR, to function as antigen presenting cells indicates that they could interact with CD4 lymphocytes to elicit antigen-specific lymphocyte responses[5].

Our results concerning the biopsy findings before and after allergen challenge in the dual responders, with respect to the correlations found between the number of activated (EG2) eosinophils before the challenge and the number of $CD3^+$ and $CD4^+$ T-lymphocytes after the challenge indeed suggest that this occurs *in vivo* in asthmatic patients.

At the time of writing, it is probably premature to define the position of eosinophils in orchestrating inflammatory responses to inhaled allergen in asthma. Although in view of their capacities pertinent to the type of inflammation seen in asthma, this component of the mucosal immune system may be the necessary link between the induction of an asthmatic response and its subsequent propagation.

In the 1970s Gökemeyer and Hargreave & co-workers established that the changes in bronchial hyperresponiveness seen after late responses occur and could even persist for weeks[6,7]. One

single bronchial challenge could produce an increase in bronchial hyperresponsiveness 2 weeks out and against an apparently normal FEV_1.

The question is: Is that a model for the way in which bronchial hyperresponsiveness is developed in the natural world? An other question is, whether the experimental challenge model provides insight into chronicity of asthma and whether the late response does duplicate chronic disease. To date, these questions are not yet solved. However, it seems of great importance to understand these phenomena, since the degree of hyperresponsiveness has been shown to be related to the severity of the disease. It has been suggested that the degree of hyperresponsiveness is correlated to the progression in lung function deterioration in chronic airflow obstruction[8-11].

Bronchial hyperresponsiveness and allergen-induced changes in bronchial hyperresponsiveness in asthma can be measured using direct and indirect stimuli.

In addition, by using direct and indirect stimuli substantial extra information can be obtained, not only by discriminating between different clinical entities, but also in respect to pathological processes involved. We demonstrated that patients with only an early asthmatic reaction may develop an allergen-induced increase in bronchial hyperresponsiveness as well, suggesting that allergen-induced increase in bronchial hyperresponsiveness as such does not discriminate between patients with only an early asthmatic reaction and dual responders.

Our studies also indicate, that bronchial hyperresponsiveness does not increase *per sé* after allergen challenge, but that it depends on the stimulus used in the procedure to measure bronchial hyperresponsiveness.

The results of this study ask for further research, especially focused on the dynamics of the inflammatory process. It seems clear now that the number and type of inflammatory cells in the submucosa and epithelial lining do play an important and possibly crucial role in allergen-induced changes in bronchial hyperresponsiveness. However, we still do not know how these cells interact, what factors or cells are responsible for the attraction of these cells to the submucosa and subsequent epithelial lining.

With the acquired and increased knowledge it is now necessary to solve these questions in order to better understand the process resulting in the clinical picture asthma.

12.4. Referenc

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