

Defects of airway smooth muscle cell function are important in asthma

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Summary

For many years asthma has been regarded as an inflammatory disease of the airway mucosa leading to bronchial hyperreactivity. Recent studies showed marked abnormalities in airway smooth muscle behaviour in patients with asthma. The pathogenesis of asthma seems to consist of airway inflammation combined with airway smooth mus-

cle remodelling. The latter pathology is linked to a lack of the ant-proliferative transcription factor C/EBP- α in this specific cell type.

Key words: asthma; airway smooth muscle; glucocorticoid receptor; C/EBP- α

Introduction

Asthma is a chronic inflammatory disease of the airways and its incidence is increasing world wide without any known reason [1–7]. Up to the mid- nineties there was an increase of asthma incidence also in Switzerland, which seems to have reached a plateau over the last ten years. With respect to geographic variation and definition asthma affects 8–18% of children and about 8% of adults. Asthma is in most cases a life long disease, which often requires continuous therapy and therefore contributes significantly to the overall health care budget [8, 9].

Asthma is characterised by chronic airway and mucosa inflammation, tissue infiltration with eosinophils, lymphocytes and mast cells [10, 11]. In addition, the adherence of the epithelial cell layer seems to be disturbed [12–14]. This pathology of the epithelium in asthma airways has been suggested to disrupt the interaction between the epithelial cell layer and the underlying connective

tissue [10–15]. As a consequence there is increased airway remodelling reflected in the thickening of the basement membrane and neo-vascularisation [10–15]. The cellular communication and interaction between bronchial epithelial cells and fibroblast like cells (myo-fibroblasts) are the focus of current research. The thickness of the connective tissue sheet underlying the basement membrane is also increased with infiltrated myofibroblasts of unknown origin and enhanced deposition of extracellular matrix, a pathology that is often described as sub-epithelial fibrosis [12–17]. In contrast to asthma fibrotic processes in chronic obstructive pulmonary disease (COPD) include fibrosis of the bronchioli [16, 17]. There is increasing evidence that smooth muscle hyperplasia plays an important role in asthma [23–38]. The major pathological differences of the bronchial wall, comparing asthmatic to non-asthmatic tissue, is depicted in figure 1.

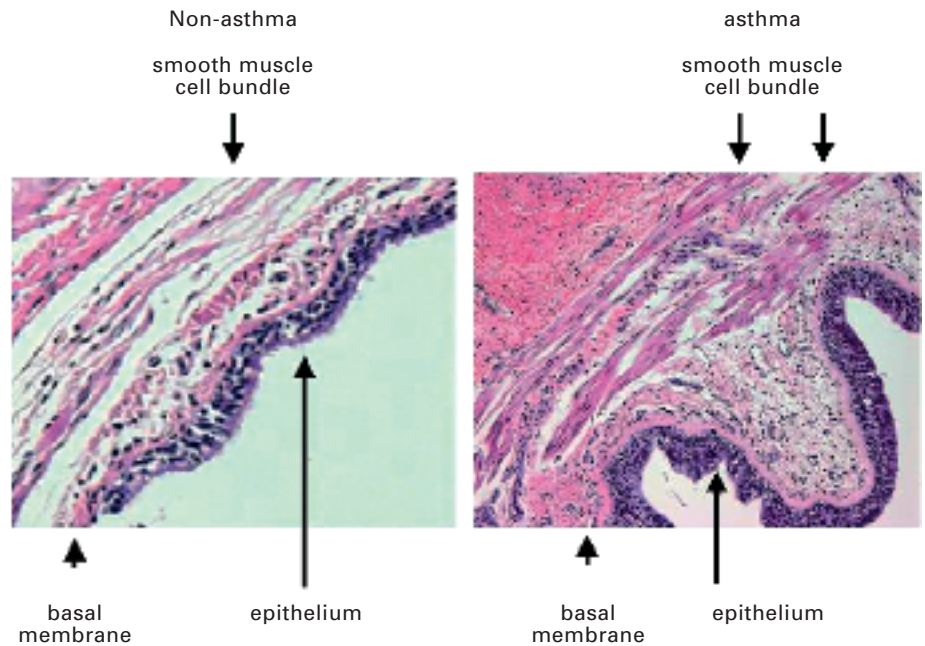
Classical hypothesis of asthma as an inflammatory lung disease

Asthma is associated with inflammation of the airway mucosa [11, 16, 19]. A significant number of asthma patients also suffer from allergic rhinoconjunctivitis [39–46]. Therefore the concept of one airway one disease was favoured [43]. The airway inflammation consists of mucosal thickening with infiltration of inflammatory cells including eosinophils and lymphocytes. Th2 cells contribute

to the aggravated inflammation in asthma since they release cytokines, which are involved in the activation of other inflammatory cells such as mast cells [47–49]. Mast cells have also been implicated in airway inflammation in asthma. Many groups have investigated their role in inflammation and tissue remodelling [50–54]. In this context it is important to note that mast cells have been shown to

Figure 1

Histology of the non-asthmatic (left panel) and the asthmatic (right panel) airway wall. In the non-asthmatic airway the bundles of smooth muscle cells is thin and consists of a few cells only, while in the asthmatic airway wall the number of muscle bundles as well as the number of smooth muscle cells per bundle increases markedly. The basement membrane in the non-asthmatic airway is a thin layer separating the epithelial cell layer from a thin layer of connective tissue formed by extracellular matrix and fibroblast like cells (myofibroblasts). In contrast in the asthmatic airway the basement membrane is significantly thickened as is the mass of the connective tissue layer.



interact with airway smooth muscle cells and modulate their function [55]. Two recent reviews by Norris [56] and Marrone et al. [57] summarise the contribution of mast cells to asthma in detail.

Major factors linked to mast cell activation in asthma are IgE, IL-4, and IL-5, which are all well known to be up-regulated in asthma patients. IgE together with IL-4 have long been known to be up-regulated in asthma patients and in their first degree relatives, but there is no study until today that could establish a clear genetic link of one of the factors to the inheritance of asthma. Furthermore, IgE inhibition by antibodies resulted in a marked down-regulation of the inflammatory symptoms but did not cure asthma and the symptoms re-appeared after cessation of anti-IgE therapy [58, 59]. Anti-IL-5 antibodies markedly down-regulated eosinophils in asthma but could not reduce the symptoms [60].

In view of asthma as an inflammatory disease of the lung the role of the Th1/Th2 ratio has been investigated intensively. Most studies however,

revealed that the shift towards Th2 cells in the lung and in circulating blood was rather associated with allergy than with asthma [61–69]. The early dominance of the Th2 cell type in asthma has been also demonstrated in childhood asthma [61, 62]. Additional studies investigated the consequences of Th2 cytokine dependent cellular signalling and its role in the inflammatory processes of asthma [65–68]. Interestingly Th2 cells interact directly with airway smooth muscle cells and respond to pro-inflammatory cytokines released by this bronchial cell type [66]. This fact suggests that Th2 recruitment could be caused by activated asthmatic airway smooth muscle cells. A similar interaction has been described for Th2 cells that interact with dendritic antigen presenting cells and both cell types activate each other in the presence of antigens [69]. In summary, the cause and the role of the Th1/Th2 cell imbalance in asthma as well as in atopy has to be re-defined and is likely to be a sign of allergic inflammation.

How to control inflammation in asthma?

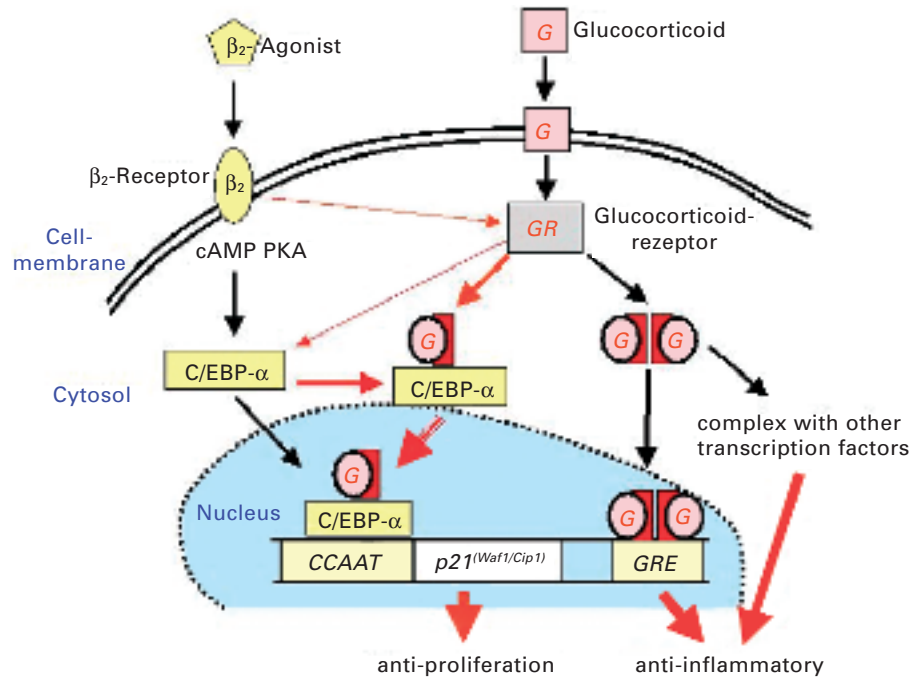
In order to control the inflammatory aspect of asthma the standard treatment includes glucocorticoids, which are among the most potent anti-inflammatory drugs available. Glucocorticoids act via their intracellular receptor, the glucocorticoid receptor, which is located in the cytosol and upon ligand binding is activated, forms a dimer and migrates into the nucleus where it acts as a transcription factor [70, 71]. The glucocorticoid receptor acts most often as an inhibitor of gene transcription but the exact conditions for this action remain to be defined [72]. In addition, the activated glucocorticoid receptor can form complexes with

other transcription factors including I κ B, C/EBPs, Stats, and AP-1 [73]. Thereby glucocorticoids may affect genes that do not contain a glucocorticoid receptor binding DNA sequence in their regulatory (promoter) region. A schema of the signalling pathway is provided in figure 2.

Under glucocorticoid therapy the inflammation of the mucosa is significantly decreased and asthma symptoms are reduced. Airway hyper-responsiveness is also significantly reduced with inhaled glucocorticoids. Therefore it is believed that airway hyper-responsiveness is a direct consequence of mucosal inflammation. However, also

Figure 2

The interaction of the glucocorticoid and β_2 -agonist signalling pathway and its consequences for the anti-inflammatory and the anti-proliferative effect of the two drugs. As C/EBP- α is missing in airway smooth muscle cells of asthma patients, the anti-proliferative effect of especially glucocorticoids cannot be effective. However, the anti-inflammatory action of glucocorticoids, mediated via the glucocorticoid receptor dimer and the GRE-promoter element as well as via the interaction of the GR with other transcription factors, is fully functional.



glucocorticoids are not capable of curing asthma and symptoms most often re-occur after cessation of treatment. From these observations it can be speculated that mucosal inflammation cannot be the only cause of asthma.

In addition to their anti-inflammatory action glucocorticoids are capable to block cell proliferation. This is of special interest since this might offer a possibility to control the increased mass of airway smooth muscle cell bundles in asthma as shown in figure 1. In an animal model it had been shown that the anti-proliferative effect of glucocorticoids involves the formation of a complex with the transcription factor C/EBP- α (figure 2), which we were able to confirm in human lung cells [74]. C/EBP- α belongs to a family of transcription factors that, like the glucocorticoid receptor, has been preserved during evolution, and both the glucocorticoid receptor and the C/EBP-isoproteins are central regulators for cell proliferation. Knock-out animal models have been tested for both the glucocorticoid receptor and C/EBP- α , but the $^{-/-}$ homozygous animals for either of the two factors did not survive birth longer than a few hours [75, 76]. We think that natural hormones (steroids) together with the transcription factors C/EBPs and peroxisome proliferator-activated receptor (PPAR) regulate cell homeostasis in the human body [77, 78]. Fibroblast and smooth muscle cell proliferation in the human lung is mainly controlled by the ratio of C/EBP- α to C/EBP- β . We reported recently that bronchial smooth muscle cells obtained from asthma patients lack the ex-

pression of C/EBP- α and therefore glucocorticoids cannot inhibit their proliferation [79]. This might explain why bronchial smooth muscle cells from asthma patients grow faster in culture than cells from COPD patients or controls [29]. The lack of C/EBP- α which was documented in the smooth muscle cells of all asthma patients of our cohort should not be confused with steroid resistant asthma, which occurs in a minority of asthma patients [73, 75].

There is increasing evidence that the combination of glucocorticoids with long-acting β_2 -agonists controls asthma symptoms better than an increase of the glucocorticoid dosage [for review see 80-82]. This clinically documented benefit of the combination of glucocorticoids with long acting β_2 -agonists opposes the initially feared "pro-inflammatory action" of β_2 -agonists [83, 84]. It was previously thought that the use of long acting β_2 -agonists initially improved clinical symptoms, followed by non-compliance of patients to inhale steroids. The latter increased again the underlying inflammation of the bronchial mucosa. We postulated earlier that the clinically significant beneficial effect of the combination of glucocorticoids with long acting β_2 -agonists is based on their molecular biological effect. When both classes of drugs are combined, they synchronise the activation of the two transcription factors, glucocorticoid receptor and C/EBP- α [85].

We demonstrated earlier that β_2 -agonists activate the glucocorticoid receptor in a ligand independent – yet to be explained – manner in human lung fibroblasts and smooth muscle cells [86]. This finding has recently been confirmed in patients after inhalation of either a glucocorticoid or a β_2 -agonist alone or in combination, as shown by immuno-histochemistry in epithelial cells [87]. How-

Table 1.

The comparison of the clinical characteristics of asthma and atopic patients shows only in asthma patients the three following parameters: sub-mucosal inflammation, Th1-Th2 shift, and an abnormality of the smooth muscle cells bundles.

	mucosal inflammation	Th1/Th2 shift	smooth muscle abnormality
healthy	negative	negative	negative
atopy	positive	positive	negative
asthma	positive	positive	positive

ever, the synchronisation of the two transcription factors might not be the only cellular signalling pathway that is modified by the combined classes of drugs [88]. The new concept of the interaction of glucocorticoids and β_2 -agonists regarding their

effect on inflammation and proliferation is shown in figure 2. In conclusion, the combination of glucocorticoids and β_2 -agonists shows a beneficial effect in asthma treatment, which can be explained via their molecular biological interaction.

The role of airway smooth muscle cells in asthma

The increased mass of smooth muscle cell bundles in the airway of asthma patients (figure 1) is an important feature, which has long been underestimated. Even in young asthma patients and in patients with mild asthma the smooth muscle cell mass is increased as recently shown by endobronchial biopsies obtained via bronchoscopy [22–28, 36, 89, 90]. This increased mass of muscle is believed to contribute to bronchial hyper-reactivity. After being able to culture for the first time bronchial smooth muscle cells of asthma patients it became evident that the smooth muscle cells of these patients behave abnormal [29, 79]. Even after long-term culture the smooth muscle cells of asthma patients proliferated at a faster rate than their controls, including COPD patients [29, 79].

Therefore inflammatory mediators present in the body can be excluded as a cause for this pathology. However, it cannot be excluded that chronic stimuli such as allergens or viruses might interfere with the C/EBP- α expression. We have shown earlier that *Chlamydia pneumoniae*, which is known to induce asthma exacerbations, affects the above described signalling pathway by “high checking” the glucocorticoid receptor and NF κ B signalling pathway [91]. Preliminary data suggest that several respiratory viruses directly alter the activity of various C/EBP-isoproteins (unpublished data). Furthermore, our group demonstrated that smooth muscle cells of asthma patients produce more connective tissue growth factor (CTGF), which could be related to the increased expression of TGF- β the increased synthesis of extracellular matrix [92, 93] and the increased synthesis of some specific extracellular matrix components [28] that contribute to airway wall thickening. In this context it is important to note that the composition of the extracellular matrix is a key regulator of airway smooth muscle cell proliferation [28, 31, 94, 95].

As described above, the ratio of the pro-proliferative C/EBP- β to the anti-proliferative C/EBP- α controls natural cell homeostasis. The increased proliferation of the airway smooth muscle is due to the lack of C/EBP- α . As described above, C/EBP- α forms a complex with the glucocorticoid receptor and is involved in the control of cell proliferation and differentiation [74, 85]. Therefore the normal anti-proliferative action of C/EBP- α is missing in smooth muscle cells of asthma patients [79]. Re-introduction of C/EBP- α by transfection experiments restored the sensitivity of the cells for the anti-proliferative effect of glucocorticoids [79]. The family of the C/EBP tran-

scription factors consists of six members ($-\alpha$, $-\beta$, $-\gamma$, $-\delta$, $-\epsilon$, $-\zeta$) in humans and they all can interact with each other and bind to the same DNA promoter sequence, CCAAT [96]. Furthermore, at least some C/EBPs can bind to the cAMP response element (CRE), thereby affecting the expression of much more genes [97]. In other cell types C/EBPs have been identified as central regulators of cell differentiation [98–100]. We speculate that the lack of C/EBP- α in the smooth muscle cells of asthma patients leads to a dysbalance with the pro-proliferative C/EBP- β [101], which explains their faster proliferation rate [29]. Interestingly, overexpression of C/EBP- β in lung epithelial cells has recently been reported in COPD patients [86]. In contrast to C/EBP- α enhanced expression of C/EBP- β and furthermore its activation is clearly linked to proliferation and de-differentiation of several cell types [100, 101]. The recent publication of Didon et al. [102] suggests that this mechanism might play a role in the pathogenesis of small airway bronchiolitis with fibroblast proliferation and collagen deposition in the small airways. The role of C/EBPs in asthma has been discussed in details earlier [103].

Furthermore, the lack of C/EBP- α and its role in cell differentiation may explain the often-described trans-differentiation of fibroblast or smooth muscle cells into so called myo-fibroblasts observed in asthma [104]. If the lack of C/EBP- α in smooth muscle cells of asthma patients leaves the cells with an “over-activity” of the remaining C/EBP- β the report of Hu et al. [88] showing that C/EBP- β regulates the expression of smooth muscle cell actin becomes important; the expression of smooth muscle cell actin is often used to differentiate between smooth muscle cells and myofibroblasts [105].

Further indications that C/EBP-isoproteins control the differentiation of fibroblast like cells into more specific differentiated cell types have been provided for C/EBP- β and the differentiation of mouse fibroblasts into adipocytes [106], and for C/EBP- δ and the transdifferentiation of myofibroblasts in the kidney [107]. The role of the different C/EBP-isoforms on the differentiation of smooth muscle cells and in asthma has therefore to be studied in more detail. The function of C/EBPs is also linked to that of the transcription factor peroxisome proliferation receptor activator, PPAR, which forms complexes with the glucocorticoid receptor and C/EBP-isoforms [77, 108]. This signalling pathway and its impact on asthma and COPD is under investigation.

Conclusion

The new findings on abnormal smooth muscle cell behaviour in asthma have challenged the hypothesis of asthma pathogenesis. Not only inflammation must be present in the asthmatic airway but also a defect of smooth muscle cell differentiation. Current and future research has to

address the underlying molecular biological mechanisms of the smooth muscle cell defect. Of special interest are the questions, how epithelial cells can control smooth muscle cell behaviour, and how allergens and viruses can influence this interaction.

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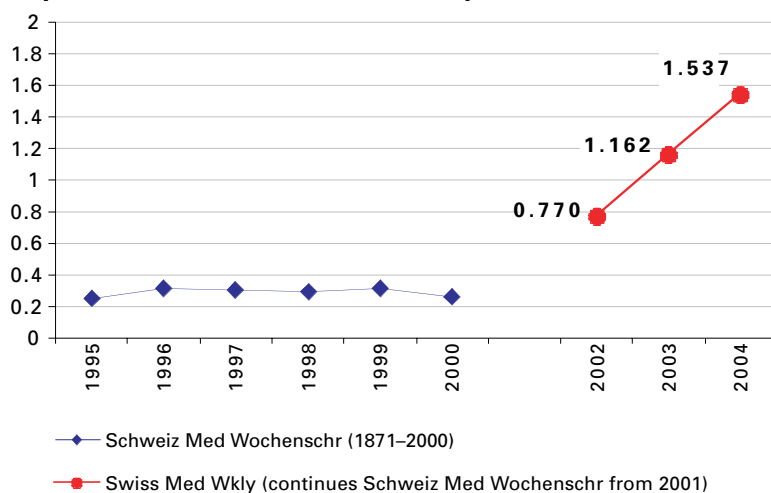
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