Eur Respir J 2011; 38: 4–6
DOI: 10.1183/09031936.00013111

EDITORIAL

Playing a dirty trick on airway smooth muscle: house dust mite does it again

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Increased airway smooth muscle (ASM) mass is a hallmark of asthma and is thought to be the main contributor to the increased airway hyperresponsiveness seen in asthmatics [1]. This increase is related to disease severity (reviewed in [2]) and caused by hyperplasia alone or in combination with hypertrophy, depending on the generation of airways [3, 4]. Why and how ASM mass is increased is incompletely understood, but *in vitro* studies have shown that ASM cells isolated from asthmatics proliferate faster in culture [5]. In addition, cultured patient cells produce more chemokines and an altered array of extracellular matrix proteins compared with those of healthy individuals [6, 7]. *In vitro* studies have shown that upon isolation, ASM cells display contractile function, but they rapidly change phenotype (*i.e.* acquire proliferative and synthetic capacity) and become noncontractile during culture under serum-rich conditions [8]. When these synthetic–proliferative cells are serum-deprived for a prolonged period of time, a small population (so-called ''hypercontractile'' cells) starts re-expressing contractile protein genes, such as *ACTA2* (encoding α-smooth muscle actin (α-SMA)) [9]. This dedifferentiation/modulation and differentiation/maturation of ASM cells is referred to as phenotype switching or phenotypic plasticity, and although this phenomenon clearly exists *in vitro*, we do not know whether this plays a role in thickening of the ASM layer.

The increase in ASM proliferation in asthma is thought to be associated with decreased levels of CCAAT enhancer protein (c/EBP)α (encoded by the *CEBPA* gene), a crucial controller of cell cycle progression, differentiation and inflammation. The biological effects of this protein are exerted by the full-length (p42) isoform and counteracted by a shorter isoform (p30), which is formed after translation of the protein. Previously, it was shown that ASM from asthmatics has reduced expression of c/EBPα, leading to reduced effects of glucocorticoids on proliferation of ASM cells [10]. Subsequently, it was shown that both the p42 and p30 isoforms of c/EBPα are reduced in asthmatic ASM cells and that culture in medium containing 5% fetal calf serum reduced the expression even more. Reduced expression was found to be the result of impaired translation and it was associated with decreased expression of the translation regulator eIF4E (eukaryotic initiation factor 4E) [11]. In addition to increased proliferation, interleukin (IL)-6 release (induced by growth factors) is increased in ASM from asthmatics and lack of c/EBPα is thought to be important for this process. Whether the change in ASM in asthmatics is innate or acquired due to exposure to external stimuli is yet to be discovered.

Bronchial provocation using house dust mite (HDM) allergens results in bronchoconstriction in allergic patients. In addition to immunological mechanisms involving immunoglobulin (Ig)E and T-helper type 2 cells, it was shown that HDM also exerts direct effects on various cell types, including protease-dependent cell detachment in epithelial cells [12] and IgE-independent activation of mast cells [13]. In a mouse model of allergic airway inflammation, it was recently shown that Toll-like receptor 4 expression on epithelial cells was sufficient and necessary for dendritic cell activation and induction of airway inflammation by HDM, suggesting an important role for structural cells in the induction of inflammation in asthma [14]. As HDM disrupts the epithelial barrier, it is plausible that HDM will also reach and affect other cell types underneath the epithelium. Exposure of rabbit ASM strips to the purified Der p 1 allergen increased airway hyperresponsiveness to acetylcholine and reduced relaxation responses to isoproterenol, and this effect was attributed to the protease activity of Der p 1 [15]. Passive sensitisation of human bronchial rings using IgE-rich serum from HDM-allergic patients increases bronchoconstriction to histamine and other stimuli, suggesting that the immunological compartment is perhaps not necessary [16]. These data suggest that the effects of HDM can be IgE-dependent and -independent, as well as protease-dependent and -independent.

In this issue of the *European Respiratory Journal* (*ERJ*), MIGLINO *et al.* [17] show that HDM extracts also affect ASM function through protease-dependent and -independent mechanisms. These investigators show that stimulation of ASM cells obtained from mild-to-moderate asthmatics with HDM results in increased cell proliferation and production of IL-6, whereas ASM obtained from healthy controls did not respond to HDM. A reduction of c/EBPα expression in response to HDM was shown specifically in asthmatic ASM cells, and was found to be mediated by two mechanisms: by a protease-independent mechanism involving increased expression of calreticulin (CRT) and by protease-activated receptor (PAR)2.

MIGLINO *et al.* [17] show an inverse relationship between c/EBPα and CRT: when CRT was inhibited using small interfering RNA, c/EBPα levels were restored. CRT may mediate some of the effects of HDM by binding to a GC-rich sequence in the *CEBPA* mRNA, resulting in inhibition of translation.

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Other functions of CRT include calcium homeostasis and quality control of protein folding. In a mouse model, it was shown that increased contractility in lung slices correlated with increased calcium in the sarcoplasmic reticulum (SR) [18]. CRT in the SR serves as a calcium chelator and may prevent release of calcium from the SR into the cytoplasm and, therefore, contraction. However, as other proteins involved in calcium release are also increased in these mouse models, the role of increased CRT in this process may be limited [19]. In addition to calcium handling, CRT also has a chaperone function, as it prevents export of misfolded proteins to the Golgi apparatus by binding to these proteins [20, 21]. Therefore, increased expression of CRT in asthmatic ASM cells may not only inhibit c/EBPα expression but also serve to prevent cells from going into endoplasmatic reticulum (ER) stress. This may be important to control ASM function, as shown in studies in which induction of ER stress in mouse ASM cells resulted in altered ECM production, which may lead to increased adhesion of inflammatory cells [22].

These findings of MIGLINO *et al.* [17] in this issue of the *ERJ* are very interesting, as they suggest that the reduction of c/EBPα expression seen in asthmatic ASM can be the result of exposure to HDM, and that this effect is independent of the immunological compartment. However, as all asthmatic patients were atopic, we can not formally exclude the possibility that their ASM cells had already been primed *in vivo* by HDM-specific IgE, although it appears unlikely that the presence of IgE persisted in culture. Recent studies have shown that the high-affinity IgE receptor (FcεRI) is present on ASM cells [23] and that sensitisation of ASM cells with IgE results in increased transcription of various CC- and CXC-chemokines [24]. Further studies are needed to investigate whether immunological mechanisms *in vivo* have contributed to the property of the cells in culture, or whether the same phenomenon is also observed in cells from asthmatic donors that do not respond to HDM or in nonatopic asthmatics. In any case, the fact that such a disease-specific cellular response persists in culture indicates the importance of, for example, epigenetic mechanisms to explain persistence of the phenotype in small groups of patients such as those studied by MIGLINO *et al.* [17] (in which genetic studies will not likely reveal differences).

Involvement of PAR2 in HDM-induced effects on ASM cells suggests that other environmental proteases (*e.g.* in mould, aeroallergens and mites) and endogenous (cell-derived) PAR2 agonists may also alter c/EBPα expression. PAR2 activation by trypsin and tryptase is known to induce ASM proliferation and contraction [25, 26], and the results of the present study indicate that in ASM from asthmatics, this is mediated by a reduction in c/EBPα. As mast cell inflammation inside the ASM bundles is found in asthmatics [27] and these cells are sources of these proteases, these cells may affect the c/EBPα expression in ASM from asthmatics.

Perhaps differentiation of ASM is also affected, as the authors show that 24 h stimulation with HDM results in partial inhibition of α-SMA expression in asthmatic ASM. This protein is important for the contractile function of ASM cells, and is often used as a contractile marker for ASM cells. Phenotype switching of ASM cells from contractile to synthetic–proliferative cells may underlie the increase in ASM bundles in asthmatics. Another protein involved in contraction, myosin light chain kinase (MLCK), has c/EBP binding sites in its promoter, suggesting that reduced c/EBPα expression could lead to reduced expression of MLCK and, therefore, reduced contractility. The current study by MIGLINO *et al.* [17], together with other studies, suggests that c/EBPα is involved in various ASM functions, including induction of inflammation, proliferation, contractility and differentiation.

In view of the role of c/EBPα in various ASM functions, it is tempting to speculate that c/EBPα could be used as a target for treatment of asthma. Introducing this protein into ASM cells from asthmatics in culture was shown to reduce proliferation rates [10]. A recent study has shown that ASM proliferation is an ongoing process in moderate-to-severe asthmatics [28], suggesting that introduction of c/EBPα may be beneficial in preventing further increases in ASM mass. The effects of HDM on ASM proliferation suggest that reducing the exposure to HDM could have positive effects; however, *in vivo* studies in mice exposed to HDM, and studies showing correlations between atopic status and c/EBPα expression are needed to further substantiate the role of HDM-induced changes in ASM cells in asthma.

The findings of MIGLINO *et al.* [17] further highlight the active role of ASM cells in airway remodelling and inflammation, and suggest that environmental factors may contribute to changes in these cells in asthmatics that persist even into the cell culture laboratory.

STATEMENT OF INTEREST

Statements of interest for both authors can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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