Food for thought - why is there more airway smooth muscle in asthma?

Brian G Oliver [1,2]

[1]School of Life Sciences, Faculty of Science, University of Technology Sydney, NSW, Australia

[2]Respiratory Cellular and Molecular Biology, Woolcock Institute of Medical Research, Sydney, NSW, 2037, Australia

Corresponding author

Professor Brian G Oliver, PhD

School of Life Sciences, Faculty of Science, University of Technology Sydney, NSW 2007; Respiratory Cellular and Molecular Biology, Woolcock Institute of Medical Research, NSW 2037, Australia

Phone: +61 2 9114 0367

Email: Brian.Oliver@uts.edu.au

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All definitions of asthma include reference to smooth muscle contraction which manifests as either episodic wheeze, breathlessness, cough, bronchospasm and/or exacerbations. Furthermore, bronchodilators are one of most effective classes of therapeutic for the management of acute exacerbations and long-term treatment of asthma. It is therefore somewhat surprising that the smooth muscle cell is often under researched in comparison to inflammatory and epithelial cells in asthma. This likely relates to the difficulty of obtaining bronchoscopic samples and the expertise to isolate and grow airways smooth cells *in-vitro*. The *in-vitro* growth of smooth muscle cells from bronchial biopsies was pioneered by Judy Black [1]. The first publication from her group characterising airway smooth muscle cells in asthma was published 20 years ago, and described increased proliferation of smooth muscle cells *ex-vivo* from people with asthma. It seems fitting that understanding the cause of this increased proliferation was elegantly investigated by Esteves and colleagues [2] in this issue of the journal.

For those outside of the field of asthma you may be wondering why proliferation of airway smooth muscle in asthma is worthy of such in-depth investigation. One of the hallmark histological features of the asthmatic airway is increased smooth muscle bulk [3] The amount of smooth muscle positively correlates with the severity of asthma [4], exacerbation frequency[5], airway reactivity to methacholine [6], and is increased in fatal versus non-fatal asthma [7]. Bronchial thermoplasty, a technique which uses heat to remove airway smooth muscle from the airways, has recently been shown to reduce smooth muscle mass by 50% and improve asthma control [8], and ten year follow-up of bronchial thermoplasty demonstrated a reduction in severe exacerbations compared to sham treatment [9]. All of these observations clearly demonstrate the importance of the smooth muscle to the pathophysiology of asthma.

The increased bulk of airway smooth muscle in an asthmatic airway could be due to a number of factors. The physical size of the individual cells could be increased (hypertrophy), the amount of extracellular matrix in the muscle is increased, or there is an increased number of cells due to increased proliferation (hyperplasia) [10, 11]. There is evidence to support all of these arguments, and interestingly there is also evidence that does not support these arguments, including studies which have found no evidence for increased proliferation [12] . Experts in the field differ on how they interpret this diametrically opposed information. When smooth muscle cells from people with asthma are investigated *in-vitro* the evidence for intrinsic hyperproliferation is consistent (e.g. [1, 13]), and when this is coupled to the known differences in phenotypes of asthma, and known differences with regards to smooth muscle hypertrophy and hyperplasia in different sized airways [14] it is highly likely that in a subset, if not in all patients with asthma, airway smooth muscle hyperplasia occurs.

If we accept that hyperplasia of airway smooth muscle in asthma occurs, the obvious question is why? *In-vivo*, many different factors contribute to cellular proliferation, including but not limited to mitogens and co-mitogens from inflammatory, mesenchymal and epithelial cells, the amount of anti-proliferative factors, extracellular matrix components (both pro and anti-proliferative), and intrinsic and/or acquired (epi)genetic factors. The study of isolated human airway smooth muscle cells *ex-vivo* allows the careful investigation of "hard-wired" pro-proliferative mechanisms. Esteves *et al* [2] took this approach too, and then applied unbiased omics to investigate pathways which contribute to the hyper-proliferative state of smooth muscle cells from people with asthma (see figure 1). The senor author of this study, Dr Trian, had previously demonstrated that mitochondrial biogenesis was partially responsible for the hyper-proliferative nature of the cells from people with asthma, and this observation

was reconfirmed and expanded in the present study. The investigators found that ATP production was increased in the cells from people with asthma, which was not the result of increased glucose uptake. To investigate further proteomics was used, which revealed that in cells from people with asthma carnitine palmitoyl transferase-2 (CPT2) a mitochondrial fatty acid enzyme involved in the metabolism of lipids was increased, and this was accompanied by increased LDL-R which facilitates entry of fatty acids into cells. Perhaps unsurprisingly, fatty acid metabolism via OXPHOS was also increased. *In-vivo* cells from people with asthma had a lower lipid content, supporting the idea of increased lipid metabolism driving ATP production and proliferation. Proof of this came from experiments in which pharmacological or genetic approaches were used to inhibit CPT2. Inhibition of CPT2 reduced proliferation in both cells from people with and without asthma, were-as inhibition of LDL-R inhibited proliferation of only cells from people with asthma. Importantly altered lipid metabolism was not altered by corticosteroids, helping to explain why increased smooth muscle bulk is not a reversible pathological feature of asthma. This is an elegant series of experiments, but it does lead to some further questions and does have some limitations.

The major limitation of the study is that the experimental approach was *in-vitro*, which is necessary for pre-clinical work such as this. The question of how to therapeutically target altered lipid metabolism in people with asthma is difficult to answer and may need new therapeutics to be developed. The investigators found that inhibiting lipid metabolism *in-vitro* had no effect on mitochondrial mass in the cells from people with asthma. Therefore, any attempt to inhibit lipid metabolism *in-vivo* might not provide long lasting effects on mitochondrial number, but if it was able to reduce ASM mass in asthma this is likely to be revolutionary for the treatment of asthma.

The broader subject of this study is the investigation of the role of mitochondria in asthma. Mitochondria do have their own DNA which is maternally inherited, but inheritance of asthma is not universal. If the mitochondria are the key to asthma pathogenesis the patchy inheritance likely relates to the fact that around 99% of mitochondrial proteins are encoded by genes located on chromosomal DNA which can be modified by epigenetic mechanisms (18). In fact, studies from Willis-Owen *et al.* and Baines *et al*., have discovered that genes encoding mitochondrial proteins such as L-2-hydroxyglutarate dehydrogenase and solute carrier family 25 member 46 protein are methylated in asthma patients (19, 20).

The study by Esteves *et al* [2] demonstrates the critical role of fatty acids in airway smooth muscle cell proliferation. This raises some important questions around diet and smooth muscle bulk. Obese asthma represents a unique phenotype of asthma, and given that there is increased fatty acids in the serum of obese patients it is easy to speculate that this should drive further airway smooth muscle hyperplasia. As far as the author knows this has not been specifically be addressed in any previous studies, but it would be interesting to investigate if any of the reported differences in smooth muscle cells *in-vitro*, or airway function *in-vivo* in people with obese asthma are related to dysfunctional lipid metabolism.

Another important and perhaps pathogenic factor in asthma is the role lipid derived mediators such as eicosanoids. Burgess *et al* demonstrated that smooth muscle cells from people with asthma were more responsive, i.e. had greater inhibition of proliferation, to PGE2 [15], but there is also evidence that in asthma there is reduced production of PGE2 [16]. Since Esteves *et al* demonstrated that there is a reduced amount of the fatty acid arachidonic acid in asthmatic airway smooth muscle cells, which is the precursor of PGE2, it would be logical to assume that the production of PGE2 is also reduced. PGE2 also has important bronchodilator

activities, and there is some evidence that virus-induced production of PGE2 is reduced in asthma [17], so it is highly likely that the consequences of increased mitochondrial lipid metabolism are likely to extend beyond proliferation.

One final comment is that the smooth muscle is much more than contractile cell, and how altered lipid metabolism, and increased mitochondrial activity affects smooth muscle cytokine secretion [18] or ECM [19] production remains to be investigated.

Figure 1. Airway smooth muscle hyperplasia contributes to increased smooth muscle bulk in asthma. Airway smooth muscle cells from people with asthma have increased fatty acid transport (via increased LDL-R) and metabolism (by mitochondrial CPT2) which drives excessive mitochondrial ATP production. This in-turn increases asthmatic airway smooth muscle cell proliferation, which contributes to the increased airway smooth muscle bulk observed in histopathological studies of asthma.

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