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COMMENTARY

An assay to evaluate the long term effects of inflammatory mediators on airway smooth muscle: evidence that TNF_α up-regulates 5-HT_{2A} mediated contraction

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Abbreviations: TNF_α, tumour necrosis factor alpha; 5-HT, 5-hydroxytryptamine

Asthma is defined as a disease categorized by airway inflammation leading to reversible airflow obstruction and bronchial hyperresponsiveness. Many patients with asthma tend to have normal lung function between attacks, at least in the early years of their disease. Because of the episodic and reversible nature of asthma early studies tended to concentrate on the acute of effects of pro-inflammatory mediators and bronchoconstrictors. We now know that the natural history of asthma is that of increased decline in respiratory function over years (Lange *et al*., 1998) and that some patients develop airflow obstruction that is poorly reversible, even to prolonged corticosteroid treatment. These changes are due to remodelling of the airway wall, comprising an increase in subepithelial connective tissue, particularly fibronectin, tenascin, and collagens I, III and V (Roche *et al*., 1989), airway smooth muscle hyperplasia and hypertrophy (Ebina *et al*., 1993) and goblet cell metaplasia, resulting in airway narrowing and mucus plugging (Aikawa *et al*., 1992). Together, all these changes result in a dramatic increase in airway wall thickness, resulting in airway obstruction and hyperresponsiveness (James, 1997).

Although airway remodelling results in significant morbidity for patients the mechanisms underlying the airway structural changes have been inferred from studies of the effects of acute inflammation. This is largely due to the absence of appropriate models in which remodelling can be studied. In the lung, a relatively small number of studies have used tissue models rather than cell culture approaches over the last 25 years. Most have employed cultured parenchymal or airway sections to examine structural changes in response to external stimuli (Mossman *et al*., 1977). Although many studies have concentrated on the acute effects of inflammatory mediators, some have used these models to examine chronic stimuli and have demonstrated responses induced over days (Dai *et al*., 2002). In recent years others have studied remodelling using whole animal models. For example, in the Brown Norway rat, recurrent allergen exposure in sensitized animals resulted in airway narrowing, increased smooth muscle mass, goblet cell hyperplasia and bronchial hyperresponsiveness (Cui *et al*., 1999). These models have helped to confirm the relationship between repeated allergen exposure and airway remodelling and have started to examine the role of mediators in the remodelling process.

In this issue of the *British Journal of Pharmacology*, Adner *et al*., (2002) describe a longer term tissue culture model to study airway remodelling. The investigators used mouse tracheal rings cultured over 32 days and characterized their model in terms of overall gene expression, smooth muscle phenotype and contractile properties. They then went on to use the model to demonstrate the effect of chronic stimulation with tumour necrosis factor alpha (TNF_α) on the contractile responses to carbachol and 5-HT.

Microscopy and basic indices of cell viability showed that the tissue remained viable over 32 days, with the only evidence of phenotypic change being epithelial metaplasia, although detailed analysis of changes in smooth muscle phenotype were not performed. Using micro-array technology, global gene expression was seen to change between fresh tissue and initial culture set up. These initial changes in gene expression are not surprising in view of the multiple differences between *in vivo* and *ex vivo* conditions; one such difference worthy of note being the use of serum free medium in the culture conditions. Serum withdrawal is known to cause changes in multiple smooth muscle gene expression (Camoretti-Mercado *et al*., 2000).

However after adaptation to culture, global gene expression seemed to change little over the following 16 days, suggesting that no major phenotypic change occurred during the culture period. That said, the demonstration, during the culture period, of a fairly constant mRNA and protein level for the specific gene under study would be a necessary confirmatory step in further studies. Functional characterization of the model, however, showed an increase in response to contractile agonists after establishment in culture which decreased over 8 days. Both carbachol (acting *via* M_3 receptors) and KCl (*via* voltage gated L-type calcium channels) showed the same effect, suggesting this to be a function of the contractile apparatus rather than of the signalling pathways. These results also imply that the amount or phenotype of the smooth muscle cells may change over time in this culture model. Similar observations in vascular smooth muscle were thought to be due to changes in calcium

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handling rather than a true phenotypic change (Lindqvist *et al*., 1999). In order to investigate true remodelling in these types of cultures, this phenomenon will need careful examination and control conditions in any further studies.

To test their model, the authors chronically stimulated their preparation with the pro-inflammatory cytokine, TNF_{α}, known to be important in asthma. TNF_{α} is secreted by inflammatory cells recruited to the airways, increased in the broncho-alveolar lavage fluid of patients with asthma and has pro-inflammatory effects upon airway smooth muscle cells in culture. A clear time- and dose-dependent enhancement in the contractile response to 5-HT was seen with TNF_{α} treatment over 8 days, demonstrating the usefulness of this model in highlighting longer term effects not observed by other model systems.

Once issues concerning smooth muscle phenotype and specific gene expression have been addressed, this model is likely to be a useful adjunct to whole animal remodelling systems, such as those in the Brown Norway rat. Adner *et al*. (2002) have gone to some lengths to characterize their model and the initial studies in this issue of the *British Journal of Pharmacology* show it holds promise. The use of the mouse was a wise choice as the mouse genome is well characterized and specific gene knockout and other transgenic strains could be used to define the role of specific proteins in this system.

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