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EDITORIAL

Emigration and immigration of mesenchymal cells: a multicultural airway wall

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Chronic inflammatory disease is accompanied by structural changes that appear to relate to disease severity and duration, and are often considered to be poorly reversible, if at all. The processes underpinning the structural changes are varied, complex and not well understood. It is clear that these structural changes contribute to tissue dysfunction.

In asthma, remodelling comprises changes in all compartments of the airway wall, with the epithelium potentially orchestrating a persistent cycle of inflammatory injury and repair [1]. The epithelium is thought to influence the underlying mesenchymal cell network through the release of transforming growth factor-β resulting in the activation of myofibroblasts that secrete excessive amounts of collagen and contribute to the burden of pro-inflammatory cytokines. Such changes in the superficial aspect of the airway recapitulate aspects of the pathogenesis of neointima formation, in which damage to the endothelium initiates a repair response that ultimately may compromise vessel function [2].

The most significant cellular component of the airway wall remodelling is an increase in the volume of airway smooth muscle (ASM), which is considered to play a dominant role in the consequences of the airway wall thickening for airway reactivity [3]. The mechanisms of the increase in volume of ASM remain ill-defined despite extensive investigation over the last 15 yrs. Hyperplasia of ASM is well evidenced, but there is also evidence for a more limited hypertrophy [4, 5]. The hyperplasia has been ascribed to proliferation *in situ* of ASM in response to growth factors and inflammatory mediators released during exacerbations of chronic asthma. Several investigations of antigen-induced airway inflammation in experimental animals, particularly those in Brown Norway rats, have demonstrated an increase in proliferation of ASM [6] resulting in an increase in the total number of ASM cells [7]. More recent evidence suggests that a decrease in the rate of ASM apoptosis may also contribute to the hyperplasia [8]. However, recent findings suggest the need to re-evaluate *in situ* proliferation as the major mechanism underlying the ASM hyperplasia. Neither we [9], nor others [10] have been able to detect increased rates of proliferation of ASM cells in biopsies using Ki67, proliferating cell nuclear antigen or cyclin D1 expression. It may be argued that in chronic asthma the rates of proliferation would be too small to detect an increase over the baseline level of cell turnover. Nevertheless, the lack of evidence for *in situ* proliferation of ASM in human asthmatic airways is consistent with the antiproliferative nature of the laminin and proteoglycan-rich extracellular matrix (ECM) surrounding the ASM cells in muscle bundles [11, 12]. An alternative mechanism for the hyperplasia was identified in a biopsy study by GIZYCKI *et al.* [13], in which an increase in the number of myofibroblasts was detected within 24 h of segmental challenge of the airways. These myofibroblasts were considered to have differentiated from pre-existing fibroblasts, or more speculatively, to be the result of ASM migration and de-differentiation to the myofibroblast. Thus, hyperplasia of ASM could be the result of migration of mesenchymal cells from environments that are more conducive to proliferation, such as the collagen-rich subepithelial region. This region would be bathed in growth factors and cytokines derived from resident fibroblasts, mast cells and epithelium, and from infiltrating leukocytes and or lymphocytes. A third, and equally intriguing, explanation of the rapid increase in myofibroblasts is offered by a study of allergen challenge, demonstrating the appearance of CD34+ procollagen expressing cells in the airway within 24 h [14]. In parallel studies *in vitro*, these cultured fibrocytes were shown to have the capacity to subsequently express α-smooth muscle actin. In a murine model of allergic inflammation CD34+, pro-collagen I expressing fibroblasts could be recruited from a circulating pool of fibrocytes [14]. Regardless of the origins of the myofibroblasts, it seems reasonable to speculate that these cells could migrate towards the muscle bundle and differentiate further towards the smooth muscle pole of the mesenchymal phenotypic spectrum. Migration in and out of the ASM bundle would be expected to generate hetereogeneity of ASM phenotype. In a recent study in the Brown Norway allergen-induced inflammation and remodelling model, chronic inflammation was associated with a decrease in content of the α-smooth muscle actin in the airway [15], consistent with the notion that some of the increase in ASM bulk in this model results from newly recruited and incompletely differentiated ASM.

The migration of mesenchymal cells, therefore, assumes importance in considering the mechanisms of airway wall remodelling and how these might be therapeutically targeted. The study by PARAMESWARAN *et al.* [16] in the current issue of the *European Respiratory Journal* adds to their earlier work showing that cys-Leukotrienes (cys-LTs) enhanced chemotactic responses to platelet-derived growth factor [17], by demonstrating that collagens III and V, as well as fibronectin, induce ASM migration by a process known as haptotaxis and that these responses are also enhanced by cys-LTs [16]. Several studies have investigated the pathways that underpin ASM chemotactic/chemokinetic responses. The upstream signalling mechanisms studied include: the activation of phosphoinositide-3-kinase, extracellular regulated kinase 1/2, src and p38 mitogen-activated kinase (MAPK) [18–21]. The latter kinase phosphorylates and activates MAPK-activated protein kinases 2 and 3, which phosphorylates heat shock

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protein 27, involved in cytoskeletal actin remodelling processes that are necessary for cell locomotion [21]. A number of these pathways are regulated by currently used anti-asthma agents and by stimuli for adenylate cyclase, such as prostaglandin E_2 [22]. In addition, glucocorticoids, such as fluticasone propionate, synergise with salmeterol to suppress chemotaxis. Our recent investigations indicates that a collagen I-rich ECM reduces glucocorticoid regulation of migration as well as proliferation [23, 24], but β_2-adrenoceptor agonists, such as salmeterol, retain their efficacy in cells exposed to collagen I rich matrices [23].

The adhesion molecules/integrins subserving migration of different mesenchymal cell phenotypes have not been extensively characterised. Further investigation of the mechanisms of cell-matrix interactions are required to define the level of selective block of mesenchymal cell migration that may be therapeutically feasible. PARAMESWARAN *et al.* [16] provide evidence of the importance of the β_1 and α_5, α_v integrins in ASM migration, whereas integrins required for circulating fibrocytes to migrate into wounded tissue remain poorly defined. The authors emphasise that monomeric ECM molecules were used in their study as previous work suggests that fibrillar ECM may suppress chemotaxis [25]. These observations are similar to those that have been made in studies on the impact of ECM on vascular smooth muscle proliferation, in which fibrillar collagen suppresses and monomeric collagen enhances proliferation of mesenchymal cells [26]. In the inflamed airway wall the possible roles of ECM protein and proteoglycan degradation products should be examined. Other features of the migration response requiring attention include the possible selectivity of signal transduction pathways subserving chemotaxis, chemokinesis, haptotaxis and haptokinesis. Identification of chemotactic/haptotactic gradients required *in situ* for directed mesenchymal cell movement could prove to be difficult, especially as these gradients would need to reverse if smooth muscle does indeed migrate off the muscle bundle to proliferate and migrate back to add to the hyperplasia. Alternatively, the chemotactic signals may be mesenchymal phenotype-selective, allowing concurrent bi-directional mesenchymal cell migration; circulating fibrocytes are insensitive to chemoattractant actions of a number of chemokines, but show chemotactic responses to the CXC7 and CCR4 ligands, stromal cell-derived factor-1α and secondary lymphoid-tissue chomokine, respectively [27]. There is increasing evidence that the phenotype of mesenchymal cells (both myofibroblasts and smooth muscle) derived from asthmatic airways and propagated in cell culture differs from that of cells derived from subjects without airways disease, showing increased ASM proliferation [28, 29] and increased airway fibroblast cytokine production [30, 31]. Thus, it will be important to determine whether the migratory potential of asthmatic derived mesenchymal cells is altered.

Whilst the initial investigations of migration of mesenchymal cells in the context of airway/lung injury and repair raise more questions than answers, there is strong circumstantial evidence to support the contention that mesenchymal cell migration plays an important role in the dysregulated injury/repair cycle that initiates and maintains the remodelled airway. Identification and enumeration of mesenchymal cells with a migratory phenotype in asthmatic airways would provide further impetus to this relatively new domain of airway wall remodelling research.

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