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EDITORIAL

The fibrocyte in pulmonary hypertension: we seek him here, we seek him there

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The fibrocyte is one of many populations of bone marrow-derived cells that can be detected in the peripheral blood and is considered to represent a mesenchymal progenitor cell [1]. The fibrocyte, as we currently understand this term, was originally described by BUCALA *et al.* [2] in 1994 and is defined by the expression of a variety of cell surface antigens, including the stem cell marker CD34, the pan-leukocyte marker CD45, and monocyte markers CD14 and CD11. The fibroblast-like nature of these cells is evidenced by their expression of collagen I, collagen II and vimentin. In normal individuals, these cells comprise <0.5% of circulating leukocytes [3]. In animal models employing surgically implanted wound chambers, it can be shown that ~16% of cells that accumulate over a number of days are putative blood-derived fibrocytes [4]. Similarly, CD34+ cells can be identified in human scar tissue, suggesting that these cells may contribute to tissue remodelling and repair [5]. Importantly, fibrocytes can be isolated and grown in culture [2]. Since these early observations, fibrocytes have been implicated in the tissue remodelling that occurs in a variety of human diseases, including idiopathic pulmonary fibrosis, asthma, pulmonary hypertension, atherosclerosis and renal fibrosis [6]. Their potential role in the pathobiology of these diseases extends beyond the accumulation of fibroblasts in scar tissue and is now thought to include regulation of fibrogenesis by the secretion of cytokines and growth factors, the production of extracellular matrix and secretion of matrix metalloproteinases [7]. In addition, they act as antigen presenting cells and promote angiogenesis [8].

The pre-clinical evidence supporting a pathogenic role for fibrocytes in fibroproliferative disease is compelling. Animal models consistently show a contribution from circulating fibrocytes. In animal models of pulmonary hypertension, as in human disease, pulmonary vascular remodelling is associated with fibroproliferative changes in the media and adventitia. Until recently, these changes were attributed to an expansion of local resident fibroblasts. Recent studies by FRID and co-workers [9–11] in the chronically hypoxic neonatal calf and in rats have shown that a proportion of the adventitial cells of pulmonary hypertensive animals are of a blood-derived fibrocyte phenotype. Depletion of bone marrow-derived monocytes prevented the adventitial remodelling. A further study in newborn mice exposed to hypoxia showed increased accumulation of bone marrow-derived cells associated with pulmonary hypertension [12]. Inhibition of chemokine receptor 4, the receptor for the stem cell mobiliser stromal-derived factor-1, significantly prevented and reversed hypoxia-induced pulmonary hypertension in neonatal mice [13]. It may be significant that these experiments were conducted in neonatal hypoxia models. Neonates may respond to a hypoxic stimulus with more exuberant adventitial remodelling. This is particularly true of the neonatal calf model, where animals develop suprasystemic pulmonary hypertension [14]. The strong demonstration of a significant bone marrow contribution to vascular remodelling is not uniform across disease models. In a rat monocrotaline model combined with unilateral pneumonectomy using green fluorescent protein-expressing chimeric bone marrow transplanted rats, SAHARA *et al*. [15] found limited evidence for a contribution of bone marrow-derived cells to the formation of the thickened media or the neointima in this model. However, those authors did confirm the presence of inflammatory cells accumulating in the adventitia of monocrotaline-treated/pneumonectomised rats. Although the authors did not characterise these cells further, the results are consistent with the above reports of fibrocyte accumulation in the adventitia of these pre-clinical models.

An important step in evaluating the role of circulating fibrocytes in human disease will be to provide evidence for their presence in the remodelled vasculature. This may prove difficult however. In human disease, the natural history of the remodelling process is probably much slower than in animal models. A feature of fibrocytes is that they tend to lose the expression of haematopoietic markers, such as CD45 and CD34, within a short time frame once incorporated into the disease tissue. This makes their identification and differentiation from resident mesenchymal cells extremely difficult in the absence of further specific markers. One avenue may be to study rapidly progressive pulmonary hypertension in neonates if tissue is available, where one might maximise the chances of seeing fibrocyte accumulation.

A further approach that could examine the hypothesis that fibrocytes are involved in human pulmonary hypertension, albeit indirectly, would be to determine whether the number or function of circulating fibrocytes is altered in patients with pulmonary hypertension. This approach has been adopted in pulmonary fibrosis, where patients have higher numbers of circulating fibrocytes [16]. Moreover, a positive correlation has been reported between the number of lung fibrocytes detected in bronchoalveolar lavage and the extent of fibrosis, and circulating fibrocyte numbers correlate with disease activity and with prognosis in idiopathic pulmonary fibrosis [16].

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In the current issue of the *European Respiratory Journal* (*ERJ*), two groups publish results on the levels of circulating fibrocytes in the peripheral blood from differing populations of patients with pulmonary hypertension. YEAGER *et al.* [17] report increased levels of fibrocytes in children and young adults with idiopathic and secondary pulmonary hypertension compared with controls. The numbers of fibrocytes correlated with pulmonary arterial pressure but not with any other clinical parameter, or length or type of treatment. It was recently shown that the prostacyclin analogue, treprostinil, inhibits the recruitment of fibrocytes to the lung in the chronically hypoxic mouse [18]. Although 10 out of 26 patients in the study by YEAGER *et al.* [17] were being treated with prostanoids, no difference was seen in the numbers of fibrocytes in these patients compared with patients on other treatments. In contrast, GAMBARYAN *et al.* [19] report a decrease in percentage of fibrocytes in adult patients with pulmonary arterial hypertension (PAH) but no difference in absolute cell counts. Here, no correlation was found between fibrocyte numbers and any clinical parameter. The results from each group of authors are not directly comparable because of differences in the methods used to define fibrocyte numbers. For example, YEAGER *et al*. [17] used CD45 and procollagen with an isotype-controlled flow-cytometric technique across the whole white blood cell gate. By contrast, GAMBARYAN *et al*. [19] used CD11b, CD34 and vimentin as markers. Less information is available on the flow-cytometric technique used but it is clear there are significant methodological differences. In addition, the different populations of subjects studied are likely to have a significant effect.

The study by GAMBARYAN *et al.* [19] also investigated the membrane expression of CD11b (an integrin, also known as complement receptor type 3 or Mac-1) on fibrocytes. CD11b was found to be upregulated on fibrocytes from PAH patients, suggesting activation. Based on this intriguing finding, it would seem worth studying further the cytokine profile of fibrocytes from PAH patients compared with controls. Important and potentially targetable differences may reside in these activated fibrocytes.

A recent Editorial in the *ERJ* elegantly summarised the current state of the field of circulating fibrocytes in vascular remodelling [7]. The idea of a pathological fibrocyte in pulmonary hypertension is an attractive therapeutic concept. As PAH involves intimal thickening and fibrosis with fibroproliferative changes in remodelled vessels, it remains important to test the traditional view that these changes reflect resident cell responses. If circulating progenitor cell fractions are significantly involved in disease, then the manipulation of these cells, how they home and differentiate, and their activation status, are valid targets. Central to this will be lineage tracing and better definitions of cell populations and hierarchies to understand how progenitors are mobilised and differentiate, and how this fits in to the overall co-ordinated response to injury. There is now a mounting body of evidence that the mobilised bone marrow response is important in vascular homeostasis and the response to injury. We now need to better characterise the cells we are working with, understand their differentiation hierarchy, and clarify how they interact with circulating and resident cells in both health and disease.

STATEMENT OF INTEREST

None declared.

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