SHORT REVIEW

From granuloma to fibrosis in interstitial lung diseases: molecular and cellular interactions

J.F. Mornex, C. Leroux, T. Greenland, D. Ecochard*


ABSTRACT: Granuloma is a feature of many chronic interstitial lung diseases, and may serve as a focus for subsequent fibrosis. Granulomas are composed of structured masses of cells of the macrophage lineage, which adopt an epithelioid aspect, interspersed with lymphocytes. They are formed around local centres of irritation. During their resolution, fibroblasts congregate around the structures and may penetrate the interior.

In many cases, granulomas can disappear without leaving lasting traces. However, especially when damage has occurred to the surrounding tissue, permanent scarring and fibrosis may occur. Both types of cell present in the granuloma are capable of secreting a number of factors influencing the accumulation and proliferation of fibroblasts, both positively and negatively.

The possible roles played by the different factors and, especially, interactions between them are discussed in the light of fibrosis formation. Possible therapeutic interventions are summarized.

Eur Respir J., 1994, 7, 779–785.

Several chronic interstitial lung diseases, such as sarcoidosis and hypersensitivity pneumonitis, are characterized by an interstitial cellular infiltrate or alveolitis, granuloma, and varying degrees of interstitial fibrosis [1–3]. Granulomatous lung diseases, like sarcoidosis, hypersensitivity pneumonitis or histiocytosis X, are typified by the formation of granulomas in the alveolar, bronchial and vascular walls [4], although they differ in histological organization. Alveolitis is thought to precede granuloma formation [1, 5]. The subsequent process of fibrosis involves the accumulation of fibroblasts and extracellular matrix around and within the granulomas, and is the main risk for evolution towards permanent pulmonary dysfunction.

Granulomas are structured masses composed of macrophage-derived cells, which assume an epithelioid aspect, and of lymphocytes. They form in response to local irritation, and traces of the irritant material may be recognizable within them [6]. They may occur in any tissue or organ. Their resolution may proceed without alteration of the tissue in which they are embedded, or may involve erosion or replacement by scar tissue. During their resolution, fibroblasts concentrate around the periphery, and may infiltrate the mass. Whether a granuloma progresses to a local or a diffuse fibrotic lesion probably depends on the extent of damage to the surrounding tissue in which the granuloma was embedded.

Over the past years, it has been demonstrated that fibrosis is under the control of inflammatory cells [7], mainly macrophages, and results from interactions between very large numbers of cells; the human lung comprises more than 10¹¹ alveolar macrophages and interstitial cells [8]. This review focuses on the molecular interactions between inflammatory cells and fibroblasts that lead to fibrosis in granulomatous lung disorders.

Macrophage mediators acting on fibroblasts

Macrophages are able to release a variety of mediators that can modulate fibroblast functions (table 1). Fibroblast proliferation can be induced by tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), (previously described as alveolar macrophage-derived growth factor (AMDFG)), interleukin-1-beta (IL-1β), platelet-derived growth factor (PDGF) and fibronectin [9–12]. Interferon-gamma (IFN-γ) stimulates proliferation in quiescent fibroblasts but inhibits the multiplication of rapidly dividing cells [11]. Interestingly, TNF-α is able, after intravenous administration, to induce alveolitis and epithelial cell damage in the absence of other factors [13]. TGF-β and IGF-1 can induce collagen synthesis [10], while IFN-γ [14] and prostaglandin E₂ (PGE₂) [15] downregulate collagen production. TNF-α and IL-1β can induce PGE₂ secretion [16], while IFN-γ decreases it [12]. Finally, the interstitium can be further modified by secreted enzymes, such as collagenase, induced in fibroblasts by TNF-α, TGF-β, IGF-1 and IL-1β whereas IFN-γ and
PGE2 decrease its secretion [10]. Alveolar macrophages are also able to modulate the proliferation of type II pneumocytes [17].

**Increased release of macrophage mediators in granulomatous lung disorders**

The above mediators have been shown to be released by macrophages in most spontaneous [10, 18] and experimental [19] granulomatous lung disorders (table 2). For instance, an increased spontaneous secretion of TNF-α by alveolar macrophages is observed in hypersensitivity pneumonitis [20] and in sarcoidosis [21–24], although in this disease other investigators only observed increased levels of TNF-α after induction by lipopolysaccharide (LPS) [25, 26]. These discrepancies are more likely to be due to differences in patient populations than to technical aspects of the assay. Increased macrophage expression of the TNF-α gene has been reported in chronic beryllium disease [27], and in an experimental model of granulomatous lung disease, where the release of TNF-α by alveolar macrophages is increased in mice 4 weeks after intranasal instillation of *Faeni rectivirgula* [28].

IGF-1 (previously AMDGF) is also released in excess by alveolar macrophages in sarcoidosis, hypersensitivity pneumonitis, histiocytosis X and berylliosis [29], as is fibronectin [30]. The level of PDGF gene expression is increased in histiocytosis X [31]. As far as IL-1 is concerned, the data are less clear. Whilst there is an increased spontaneous release of IL-1 by alveolar macrophages 2 weeks after intranasal instillation of *Faeni rectivirgula* in mice [28], and in hypersensitivity pneumonitis in human [20], the release of IL-1 has been a matter of controversy in sarcoidosis. It is known that the epithelioid granuloma cells and alveolar macrophages express cytoplasmic IL-1 [32], and that, in experimental pulmonary granulomatous disorders, both giant cells [4] and epithelioid cells produce IL-1 [33]. The spontaneous [34] or LPS-induced [35] release of IL-1 by alveolar macrophages in sarcoidosis was initially thought to be increased. But the levels of IL-1β messenger ribonucleic acid (mRNA) is not increased in the alveolar macrophages in sarcoidosis patients when compared to controls [36], in keeping with data showing that alveolar macrophages per se express the IL-1 gene poorly [37].

Macrophage adhesion and activation are early events, as shown *in vitro* [38] and *in vivo* [39] after *Schistosoma mansoni* infection, during leprosy [40], in tuberculosis [41, 42], and after visna maedi virus infection in sheep [43, 44].

Considering only the fibrosis-inducing mediators released by alveolar macrophages, it is clear that most of them are spontaneously released in excess in granulomatous lung diseases (table 2). Similarly, the cytokine gene expression in leprosy granulomas shows increased levels of IL-1, TGF-β and granulocyte macrophage colony stimulating factor (GM-CSF) mRNAs [45].

**Interaction with lymphocytes and expansion of mononuclear phagocytes**

The degree of structural formation of granulomas varies in different conditions, reflecting a difference in the organizing activity of the cells involved. Cellular adhesion

---

**Table 1. – Effect of the different macrophage mediators on fibroblast metabolism**

<table>
<thead>
<tr>
<th>Cell proliferation</th>
<th>Collagen synthesis</th>
<th>PGE2 secretion</th>
<th>Collagenase secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IGF-1/AMDG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGE2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : upregulation; -: downregulation; +/-: variable results according to experimental conditions. PGE2: prostaglandin E2; TNF-α: tumour necrosis factor-α; TGF-β: transforming growth factor-β; IGF-1: insulin-like growth factor-1; AMDGF: alveolar macrophage-derived growth factor; IL-1β: interleukin-1β; PDGF: platelet-derived growth factor; IFN-γ: interferon-γ.

---

**Table 2. – Increased release of the different macrophage mediators acting on fibroblast metabolism associated with granulomatous lung disorders**

<table>
<thead>
<tr>
<th></th>
<th>TNF-α</th>
<th>IGF-1</th>
<th>IL-1β</th>
<th>PDGF</th>
<th>Fn</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcoidosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Experimental HP</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histiocytosis X</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Berylliosis</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Fn: fibronectin; HP: hypersensitivity pneumonitis. For further abbreviations see legend to table 1.
molecules participate in the localization of macrophages and in antigen presentation by them [46], and increased expression of CD11b and CD54 by alveolar macrophages has been observed in sarcoidosis [46, 47], and hypersensitivity pneumonitis [48]. Another potential mediator is basic fibroblast growth factor (bFGF), which is known to modify cell adhesion properties [49], and is produced by macrophages.

These molecules play a crucial role in the structural formation of tissues, and the level of their expression is a probable factor in the organization of a granuloma [6], and hence the type of fibrotic lesion which may follow its unsatisfactory resolution. The level of vitronectin, a mediator involved in cell adhesion, in bronchoalveolar lavage is consistently elevated in sarcoidosis [50], and in hypersensitivity pneumonitis [51]. The number of macrophages may also be increased by local proliferation within the alveolar spaces [52], and in the granulomas [32].

**Modifications of fibroblast metabolism**

Modifications of fibroblast metabolism, probably due to the action of these mediators, have been observed in most of these disorders. Fibroblast activation is strongly suggested by the presence in bronchoalveolar lavage of an increased level of procollagen III terminal peptide in sarcoidosis [53], and in hypersensitivity pneumonitis [54, 55], and of hyaluronic acid both in sarcoidosis [56, 57], and symptomatic hypersensitivity pneumonitis [54]. Thus, most interstitial lung diseases with granuloma formation are characterized by an increased spontaneous release by alveolar macrophages of cytokines able to stimulate fibroblast metabolism, and indeed, increased fibroblast metabolism can be demonstrated in most of these natural disorders. Increased lung collagen is also observed in experimental hypersensitivity pneumonitis [58].

**Role of lymphocytes**

Lymphocytes are able to modulate fibroblast function [59, 60], but whilst they stimulate proliferation in idiopathic pulmonary fibrosis, they have a negative effect in hypersensitivity pneumonitis [61]. They can modulate the influx of monocytes to the alveolar spaces and granulomas, in concert with immature monocyte-like macrophages, as shown in sarcoidosis by phenotype [62], and function [63]. Finally, CD4 positive T-cells accumulate during granuloma formation in tuberculosis [64], and sarcoidosis [65].

**Differences in the pattern of fibrosis**

The pattern of fibrosis clearly differs between the various granulomatous diseases. They can be associated either with a diffuse interstitial lung fibrosis, as in the case of hypersensitivity pneumonitis, or a focal perigranulomatous fibrosis as in sarcoidosis.

The cells constituting a granuloma can and do secrete factors that attract and stimulate fibroblasts. They can also secrete factors which limit fibroblast activity and which reabsorb the collagen produced. Not all granulomas lead to fibrosis, and not all fibrosis is preceded by a granuloma. Macrophages and T-lymphocytes can secrete fibroblast-modifying factors in contexts other than granulomas. Diffuse fibrosis could result from the expression of the same activities by cells which have not organized into a granulomatous structure. The precipitating factor may be diffusely present in the organ, or the responding cells may not be induced to organize, or may respond differently to stimulatory mediators [66, 67]. Multiple factors are probably involved in these differences in the fibrotic process:

1) The level of release of mediators by alveolar macrophages may differ. For example, the level of release of both IGF-1 [29], and fibronectin [30], is lower in sarcoidosis than in idiopathic pulmonary fibrosis. Similarly, the level of expression of PDGFβ by alveolar macrophages is lower in sarcoidosis than in idiopathic pulmonary fibrosis [68].

2) Inhibitory mediators can be released, for instance, IFN-γ released during sarcoidosis can down-regulate the activation of fibroblasts [69, 70], and, indeed, changes in fibrin structure, indicated by increased levels of D dimer in bronchoalveolar fluid, suggest a diminution of fibrinogenesis in sarcoidosis [71].

3) The mediators can interact. Both IL-1β or TNF-α stimulate the proliferation of fibroblasts; however, when added together to cell cultures they inhibit this proliferation [72]. IL-1β potentiates, but PGE2 blocks the proliferation induced by the association of fibronectin and IGF-1 [73]. TNF-α and IFN-γ added together block the stimulatory effect of TGF-β on collagen production [74]. Some mechanisms underlying these interactions have been proposed: for example, PDGF induces IL-1 receptors on fibroblasts [75], and IL-1 induces the expression of PDGF [76]. It should, however, be noted that all the preceding tests have been performed in vitro, and the results of similar associations in vivo are unknown.

4) Fibroblasts can modulate other cell types. There are clear interactions between macrophages and fibroblasts. Fibroblasts can release mediators, including cytokines, that can in turn act on macrophages and other fibroblasts. TNF-α and IL-1β [16], or PDGF [4], can induce fibroblasts to release PGE2, which inhibits macrophages and fibroblasts. In vitro, fibroblasts stimulated by TNF-α expressed monocyte chemoattractant protein-1 (MCP-1-huJE), able to attract and activate monocytes [77], and macrophage inflammatory protein-2 (MIP-2), a proinflammatory cytokine [78]. In vivo, lung cells expressed high levels of mRNA for MCP-1-huJE in experimental pulmonary granulomatosis [79], and of mRNA for MIP-1α and MIP-2 after experimental exposure to silica [78].

5) Interactions with the extracellular matrix occur and play a role in controlling these interactions. Extracellular matrix can locally concentrate cytokines, such as TGF-β and PDGF. Collagen and collagen fragments stimulate alveolar macrophages to release IL-1β, IL-6 [80], and neutrophil chemotactic activity [81]. Additionally, complex structures, such as acellular sarcoid granulomas, can directly activate fibroblasts to release collagenase and stromelysin [82].


60. Prakash S, Wyler DJ. Fibroblast stimulation in schisto-
59. Fulmer JD, Flint A, Law DE. Experimental granulomatous
58. Bjermer L, Eklund A, Blaschke E. Bronchoalveolar
56. Teschler H, Thompson AB, Pohl WR, Konietzko N, Rennard SI, Costabel U. Bronchoalveolar lavage pro-
collagen-III peptide in recent onset hypersensitivity pneumonitis: correlation with the extracellular matrix
36. Raines EW, Dower SK, Ross R. Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. Science 1989; 243: 393–396.
29. Rennard SI, Bittermann LA, Grimaux JA. Interstitial collagenase (MMP-1), gelatinase (MMP-2) and stromelysin (MMP-3) released by human fibroblasts cultured on acellular sarcoid granulomas (sarcoid matrix complex, SMC). Matrix 1989; 9: 382–388.
28.  


