Peritoneal inflammation and fibrosis in peritoneal dialysis

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Abstract

Sustained peritoneal inflammation, secondary to peritoneal dialysis (PD) treatment, induces a reparative process. This process is carried out by submesothelial fibroblasts and is responsible for many of the peritoneal structural anomalies observed during PD. Peritoneal fibrosis, mesothelial loss, vasculopathy and angiogenesis are common consequences of PD treatment. Evidence of fibroblastic heterogeneity

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has been well documented over the past years with respect to their origin, function and molecular phenotype. Among the different fibroblastic phenotypes in this setting, the myofibroblast -characterized by partial smooth muscle differentiation- is the most important one. Fibroblasts can originate from resident stem cells, mesothelial cells (through epithelial-to-mesenchymal transition) and bone marrow or circulating cells. This heterogeneous cell origin reveals new pathogenic mechanisms and offers novel therapeutic possibilities. Fibroblasts participate in extracellular matrix synthesis and inflammation which makes them responsible for the peritoneal fibrosis and modulation of the inflammatory response observed with variable intensity in PD patients. In addition, fibroblasts are an important source of vascular endothelial growth factor, which is responsible for peritoneal angiogenesis, increased vascular permeability and subsequent loss of ultrafiltration. The contractile, myofibroblast phenotype would correlate with the progressive peritoneal visceral encapsulation observed in some forms of peritoneal sclerosis. Another potential area of pathogenic and therapeutic interest is fibroblast apoptosis. Disappearance of activated fibroblasts and myofibroblasts may prove to be important in successful healing. There is accumulative evidence that in some forms of pathologic fibrosis activated fibroblastic cells escape apoptosis. The susceptibility of peritoneal fibroblastic subtypes to apoptosis is not established and this might explain the autonomous progression of fibrosis observed in some patients.

Introduction

Peritoneal dialysis (PD) is an alternative to hemodialysis for the treatment of end-stage renal disease. The peritoneal membrane acts as a permeable barrier across which ultrafiltration and diffusion take place. The morphology of the peritoneum is simple, with a single layer of mesothelial cells that covers a submesothelial region composed of connective tissue with few fibroblasts, mast cells, macrophages and vessels (1). One of the most important issues in PD is the long-term preservation of the peritoneal membrane function. The bioincompatibility of dialysis fluids, episodes of peritonitis and the uremic status are considered the main etiologic factors leading to the functional decline of the peritoneal membrane (2,3). They induce a sustained situation of peritoneal chronic inflammation that can be exacerbated periodically by acute episodes (peritonitis). Closely linked to the inflammatory response is the reparative process. Its activation is responsible of many of the structural abnormalities of the peritoneal membrane. The most important ones are loss of mesothelial cells, submesothelial fibrosis, neoangiogenesis and hyalinizing vasculopathy (4-6). Such alterations are considered the major cause of ultrafiltration failure and loss of the dialytic capacity of the peritoneum. There are two different pathologic forms of PD-related fibrosis. The most common is
simple peritoneal sclerosis, which appears in almost all patients. The degree of fibrosis is usually mild and shows a relation with time on dialysis (1). Simple fibrosis ceases when the patient is transplanted or shifted to hemodialysis. On the other end of the spectrum is sclerosing peritonitis. It is an uncommon form of sclerosis that evolves rapidly with intense fibrosis, inflammation and fibrin deposits. It is a life threatening condition that in many cases evolves to visceral encapsulation. Fibrosis progresses even, or specially, if the patient is removed from PD (7). On both clinical situations, simple fibrosis and sclerosing peritonitis, the reparative process induces important structural modifications of the peritoneum. In this review we will focus on the main cellular element responsible of the reparative-fibrotic process: the fibroblast. Recent studies reveal important new information regarding fibroblastic origin, mechanisms of activation and pathogenic capacity. We will review their role in peritoneal pathology and new information obtained from other models of fibrosis.

Fibroblast phenotypes

Fibroblasts represent a dynamic population of cells showing functional and phenotypic diversity. During the last years, numerous different molecules have been reported to be expressed by tissue fibroblasts including peritoneal ones. Not all fibroblasts express a similar molecular profile at the same moment. This heterogeneity probably reflects their different origin, activation status and functional capacity. In addition, some studies show that fibroblasts might be heterogeneous between organs and tissues. These “tissue-specific” fibroblasts show functional and phenotypic diversity. Therefore, results obtained using a specific type of fibroblast cannot be automatically extrapolated to others.

Among the different molecules associated with different fibroblastic phenotypes, those related to smooth muscle differentiation are of the greatest importance. The term myofibroblast defines a cell with intermediate features between a fibroblast and a smooth muscle cell (smooth muscle-like fibroblast). From an immunophenotypic perspective, they are defined by the expression of \( \alpha \)-smooth muscle actin (SMA). Myofibroblasts were initially described in the granulation tissue of a cutaneous model of wound repair (8,9). Since then, they have been reported to play important roles in almost all situations related with repair and fibrosis in human pathology (10). Their capacity to synthesize extracellular matrix elements, growth factors, cytokines and participation in the inflammatory response, as well as their contractile properties, makes them the most important fibroblastic phenotype. In fact, myofibroblasts are considered the “reference” fibroblast phenotype to which all others must be compared (11).

Myofibroblasts are present neither in the normal peritoneum nor in the peritoneum from uremic non-PD patients (12). In contrast, they can be easily
detected in many patients undergoing PD treatment (12-15). Thus, we have observed them in very early stages of PD preceding the morphologic appearance of fibrosis (16). Within the peritoneum they have a peculiar tissue distribution with greater density in the submesothelial superficial zone (16). It seems that not all fibroblasts are capable of transforming into myofibroblasts. Using human fibroblasts it has been demonstrated that this capacity is limited to certain subtypes and related to their tissue origin. Thy-1 is a glycoposphatidylinositol-linked outer membrane glycoprotein heterogeneously expressed in fibroblasts from many tissues (17). Its function is still unknown but it can separate fibroblasts into Thy-1+ and Thy-1- phenotypes with different functional and phenotypic capacity. In a study performed in myometrial and orbital fibroblasts, only those showing a Thy-1+ phenotype were capable of myofibroblastic differentiation after treatment with TGF-β (18). Recent studies with lung fibroblasts demonstrated that the Thy-1- phenotype has an enhanced proliferative capacity in response to fibrogenic growth factors. Thus, compared to normal controls, Thy-1 -/- mice show greater fibrosis and myofibroblastic differentiation after bleomycin induced pulmonary injury (19). Similarly, in humans with idiopathic pulmonary fibrosis, no Thy-1 staining was seen in fibroblastic foci. These results contrast with those of normal lung fibroblasts that are predominantly Thy-1+ (19). These findings suggest that the presence of Thy-1 expression on lung fibroblasts limits the development of fibrosis. Since phenotypic and functional properties of fibroblasts also depend on their tissue origin, specific studies of Thy-1 expression are needed in peritoneal fibroblasts.

The peritoneal myofibroblastic phenotype seems to correlate with the expression of heat shock protein (HSP) 47 (20). HSP are chaperons induced by stress (heat). Their function is to rescue “stressed” proteins from misfolding, allowing a correct synthesis. HSP-47 is a collagen-specific chaperon that is essential for biosynthesis and secretion of collagen. In contrast to resting peritoneal fibroblasts, those from PD patients expressed HSP. Such expression was higher in patients with ultrafiltration loss (20). In correlation with α-SMA, HSP-47 is not expressed in uremic, non-PD patients (20,21). The importance of HSP-47 chaperon was demonstrated using a rat model of peritoneal fibrosis. The group of animals treated with antisense nucleotides against HSP-47 did not develop peritoneal fibrosis (22).

Another interesting molecule expressed by fibroblasts is CD34, an antigen characteristic of bone marrow hematopoietic stem cells. CD34 is also expressed in numerous resident fibroblastic cells distributed through the organism (16,23). For some authors, tissue CD34+ fibroblasts are closely related to circulating CD34+ fibrocytes and reflect a bone marrow origin (24). Circulating fibrocytes were described by Bucala et al as a new subtype of circulating leukocytes (25). It comprises 0.1-0.5% of leukocytes and express
collagen I, CD45RO, CD13, CD11b, CD34, CD86 and MHC class II. In culture, they have a spindle shape and transform into myofibroblasts when exposed to TGF-β. Normal peritoneal fibroblasts show an intense expression of CD34 that diminishes under situations of fibrosis (16). A similar loss of expression in relation with fibrosis has been described in other tissues (24,26,27). Its significance is unknown but seems to correlate with the appearance of the myofibroblastic phenotype. Another molecule whose expression is related to the myofibroblastic phenotype is osteopontin. This protein participates actively in dystrophic calcification, a phenomenon known to occur in the peritoneum of PD patients, mainly in those who develop sclerosing peritonitis. As opposed to normal controls, osteopontin is expressed by submesothelial fibroblasts of PD patients and correlates with that of α-SMA (28).

Different reports reveal that human fibroblasts obtained from patients with pulmonary fibrosis show low levels of expression of cyclooxygenase-2 (COX-2) (29,30). Fibroblasts from the fibrotic lung fail to induce COX-2 expression in response to different stimuli. This will result in no up-regulation of prostaglandin E₂ in response to TGF-β and no antiproliferative effect. Prostaglandin E₂ also inhibits collagen production. These findings contrast with those in fibroblasts obtained from human peritoneal adhesion foci (fibrosis), which show a high COX-2 expression (31). Meanwhile, normal peritoneal fibroblasts showed no expression of this enzyme (31). Moreover, this COX-2 up-regulation is accompanied by that of prostaglandin E₂, indicating a possible inflammatory response, and this provides the possibility of therapeutic modulation (32). Specific studies of COX-2 expression are needed in peritoneal fibroblasts from PD patients.

We have also learned from the pulmonary fibrosis model that a subset of active fibroblasts express telomerase (33). This enzyme is responsible for maintaining the length of the telomeres, which are essential for continuous cell division. Under normal conditions, cellular aging is associated with shortening of the telomeres. Such shortening finally results in cellular instability and death. Indeed, telomerase expression and length preservation of the telomeres are a common characteristic of malignant neoplastic cells. Telomerase activity has not been evaluated in peritoneal fibroblasts. If overexpressed, an increase of fibro-myofibroblasts survival resulting in a pro-fibrogenic situation would follow.

There are still no comparative studies between the fibroblastic phenotypes in simple peritoneal sclerosis and sclerosing peritonitis. The irreversibility that characterizes sclerosing peritonitis reveals the existence of antiapoptotic and proliferative signals that lead to a situation of autonomous fibro-myofibroblastic activation. Differences on the phenotype of fibroblasts will help us to establish the pathogenic importance of each molecular system involved. Up to now the myofibroblastic phenotype seems to be the most aggressive one. Its relation with other molecular systems such as Thy-1, heat
shock proteins, CD34, osteopontin, COX-2 and telomerase are still not clear.

**Origin of fibroblast phenotypes**

The origin of tissue fibroblasts has been largely overlooked, so that their lineage is not fully elucidated. There is now evidence supporting that fibro-myofibroblasts might originate from different sources. Firstly, they may differentiate from resident tissue stem cells or fibroblasts (10,34,35). Secondly, they can originate from nearby epithelial cells through a process known as epithelial-mesenchymal transition (EMT) (36). Finally, the bone marrow and circulating cells may be responsible for the production of fibro-myofibroblasts circulating in the blood stream to their final tissue destination (37). EMT was initially described as an important source of fibro-myofibroblasts in the renal model of fibrosis (38-40). This process has also been involved in the formation of ocular cataracts and, recently, in pulmonary fibrosis (41,42). A similar mechanism involving mesothelial cell has been demonstrated to occur in the peritoneal membrane (43-45). Part of the evidence for EMP comes from “in vivo” studies showing that a subset of submesothelial fibroblasts express some mesothelial markers (16,44,45). This phenomenon was known to occur in the pleura, where it was thought to represent a regenerative mechanism of mesothelial cells from submesothelial stem cells (46). Cytokeratins, ICAM-1, calretinin and E-cadherin, which normally are restricted to mesothelial cells, can be detected in a small subset of submesothelial fibroblasts from PD patients. These fibroblasts are not present under normal conditions but can be seen in other situations of peritoneal fibrosis (47). The “in vivo” proportion of myofibroblasts originated from mesothelial cells remains to be established. Similarly, the dynamics in which EMT takes place has yet to be defined. Most likely, it is not a uniform process, and several factors may result in variations of its frequency. Among others, the number of mesothelial cells susceptible of transition and peritonitis episodes must have an influence on EMT. In this sense, we have observed that tissue samples with acute peritonitis revealed the highest indicators of EMT (47). In addition to highlighting the pathogenetic role of mesothelial cells, EMT is an important target for new therapies (48,49).

Both, the bone marrow and circulating fibrocytes have been demonstrated to be another source of fibroblasts. For instance, in liver cirrhosis, bronchial asthma, and pulmonary fibrosis a variable subset of fibro/myofibroblasts derive from the bone marrow (37,50-53). A similar bone marrow origin has been demonstrated for some of the peritoneal myofibroblasts that appear in granulation tissue secondary to the peritoneal implantation of a foreign body (54,55). The great similarity of the fibrogenic process, regardless of the causative agent, permits us to suspect that a similar bone marrow contribution
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May exist in PD-induced fibrosis. This is a challenging concept that raises numerous pathophysiologic questions and offers novel therapeutic approaches. It seems important to establish to what extent they contribute and if there are differences between the situation of simple peritoneal fibrosis and sclerosing peritonitis.

**Functions of fibroblast phenotypes**

The most important and well-known function of fibro-myofibroblasts is the production of extracellular matrix elements that will result in the appearance of fibrosis (10). They also play a role in angiogenesis, modulation of the inflammatory response and tissue contraction. This heterogeneous functional capacity is directly related to the peritoneal pathology secondary to PD treatment (sclerosis, neoangiogenesis, peritonitis, and encapsulation). In almost all fibrotic disorders, the myofibroblast has been identified as the major cellular component involved in collagen deposition, and its presence correlates with disease progression (10,56-58). In addition to collagen, these cells produce other extracellular matrix molecules and growth factors. The latter induce further cell proliferation, terminal cellular differentiation and mobility. Among these factors, due to its important role in peritoneal pathology, it is worth mentioning TGF-β. The role of fibro-myofibroblasts on the genesis of fibrosis is a complex process reviewed elsewhere (10). The importance of fibroblasts and TGF-β in the genesis of peritoneal fibrosis has been well described in experimental studies using animals models. The depletion of peritoneal fibroblasts acts preventing morphologic and functional modifications of the peritoneum (56). Adenovirus-mediated gene transfer of TGF-β1 causes peritoneal fibrosis and angiogenesis (59).

During the last years, both neoangiogenesis and vascular endothelial growth factor (VEGF) have been recognized as important hallmarks of peritoneal pathology (60-63). VEGF induces neoangiogenesis and increases endothelial cell permeability, both of which are potential mechanisms responsible for peritoneal increased solute transport and ultrafiltration loss. Both mesothelial and endothelial cells were considered the main source of peritoneal VEGF, but several studies demonstrate that peritoneal fibro-myofibroblasts also synthesize VEGF (12,56,63). Indeed, we have observed that fibro-myofibroblastic peritoneal cells resulting from EMT of mesothelial cells produce a higher amount of VEGF than normal mesothelial cells, supporting the pathologic importance of fibro-myofibroblasts and the relevance of EMT as a pathogenic mechanism (64).

Myofibroblasts are known to play a major role on the inflammatory response. They are capable of increasing or downregulating this response by the secretion of soluble mediators (10). Based on in vitro observations,
peritoneal fibroblasts contribute to the cytokine network by secreting immunologically active molecules into the peritoneum (65). For instance, fibroblasts can express monocyte chemoattractant protein-1, resulting in macrophage chemotaxis (66). In an in vivo model, the deletion of fibroblasts reduced the number of infiltration macrophages, suggesting that the former cells are involved in macrophage recruitment (56). Numerous profibrogenic cytokines and fibroblast growth factors produced by macrophages may lead to a vicious cycle that perpetuates fibrogenesis. The deletion of fibroblasts can interrupt this cycle. The role of myofibroblasts as a source of inflammatory mediators is particularly interesting in the PD-induced model of peritoneal fibrosis, since normally the cellular scenario exhibits few inflammatory cells.

The last of the myofibroblast functions that will be mentioned is the contractile capacity (10). An important feature of fibrotic tissue is that it has different mechanical properties (a decreased compliance and increased contractility), compared to those of normal tissue. Again, the myofibroblast phenotype helps to explain these different mechanical properties. Therefore, expression of α-SMA and other similarities to smooth muscle, correlates with the contractile phenotype as demonstrated in wound contraction in vivo, and the contraction of collagen gels in vitro (10). Concerning peritoneal pathology, some forms of sclerosing peritonitis are characterized by progressive encapsulation of the abdominal viscera, mainly the small bowel. Such encapsulation reflects tissue contraction, and the myofibroblast emerges as the most likely cellular phenotype responsible for it.

From these aforementioned findings, it follows that activated fibroblasts and myofibroblasts may be responsible for a great part of the peritoneal structural anomalies secondary to dialysis. In addition, EMT has enhanced the pathogenetic role of mesothelial cells in this setting, since they can transform into myofibroblasts. When compared to other situations of peritoneal fibrosis, that induced by PD shows some morphologic peculiarities (47). PD-related fibrosis, at least in advanced stages, is characterized by loss of mesothelial and fibroblastic cells (“cellular dessert”). The reason for this cell loss is not clear but may relate to the toxicity of dialysis fluids. It is interesting to speculate if such cellular loss protects the peritoneum itself from further damage. The proliferative component of the reparative process that normally results in fibroblastic hypercellularity, may be partially attenuated by the cellular toxicity induced by PD fluids. This will result in a lower number of fibro-myofibroblasts and less fibrosis, VEGF production, etc. As will be mentioned below, the escape from apoptosis is a mechanism of fibro-myofibroblastic survival that perpetuates fibrosis. PD fluids have proved to induce apoptosis in mesothelial and other peritoneal cells. At least theoretically the apoptosis of activated fibroblasts and myofibroblasts will help to preserve peritoneal
integrity. This could be considered as a desirable toxic effect of PD fluids.

**Destiny of fibroblast phenotypes**

Once the causative agent of cellular and tissue injury is removed, the activator signals of inflammation and reparative fibrosis gradually disappear. What happens then to activated fibroblasts, myofibroblasts and the newly formed extracellular matrix? During the course of normal wound healing, myofibroblasts disappear, possibly by apoptosis. In contrast, when there is abnormally exaggerated wound healing, myofibroblasts persist (67,68). Lets extrapolate this situation to that of PD-induced fibrosis. This model is characterized by a continuous chronic injury with occasional episodes of acute exacerbation (peritonitis). Peritoneal functional and morphologic improvement has been obtained after cessation of PD (“peritoneal rest”) (69-72). These observations suggest reversibility of the fibrotic process and would be similar to a normal wound tissue reaction. The situation of abnormal cutaneous scarring shows similarities to the progression of fibrotic disease seen in liver cirrhosis, end-stage kidney disease, pulmonary idiopathic fibrosis and sclerosing peritonitis. All these situations are characterized by irreversible progression of fibrosis despite the elimination of the causative ethiologic agent. This autonomous behavior resembles that of neoplastic cells. One of the characteristics of the latter is their capacity to escape from apoptosis, and a similar capacity has been described in fibro-myofibroblasts obtained from fibrotic organs. Studies from lung fibroblasts suggest the pro-apoptotic activity of nitric oxide. However, in the presence of TGF-β, myofibroblasts are protected against nitric oxide-induced apoptosis (73). Peritoneal fibroblasts obtained from adhesions are shown to resist apoptosis due to anti-apoptotic effects mediated by hMet1-e and chondroitin sulfate proteoglycan 2 and downregulation of insulin-like growth factor binding protein-3 precursor (74). Other studies in the same fibroblast population reveal a higher proliferation and reduced apoptosis due to an altered ratio of bcl-2 and bax expression (75). There is a considerable interest in establishing the in vivo signals of apoptosis and what fibroblast phenotypes become capable of escaping cell death. From the comparison of the clinico-pathologic forms of peritoneal fibrosis it seems possible that fibroblasts from sclerosing peritonitis are partially protected from apoptosis. Most of this revision has been focused not on resting fibroblasts, but on activated fibro-myofibroblasts. Probably, most of them are prone to cell death via apoptosis. However, the reversibility of myofibroblastic transformation should be also considered. In this sense, the plasticity of fibroblasts has been well documented and the possibility of mesenchymal to epithelial transition has been documented in renal fibroblasts (76).
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