Fibroblast differentiation in wound healing and fibrosis

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Running title:
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Abstract

The contraction of granulation tissue from skin wounds was first described in the 1960s. Later it was discovered that during tissue repair, fibroblasts undergo a change in phenotype from their normal relatively quiescent state in which they are involved in slow turnover of the extracellular matrix, to a proliferative and contractile phenotype where they are termed myofibroblasts. These cells show some of the phenotypic characteristics of smooth muscle cells and have been shown to contract in vitro. In the 1990s, a number of researchers in different fields showed that myofibroblasts are present during tissue repair or response to injury in a variety of other tissues, including the liver, kidney and lung. During normal repair processes, the myofibroblastic cells are lost as repair resolves to form a scar. This cell loss is via apoptosis. In pathological fibroses, myofibroblasts persist in the tissue and are responsible for fibrosis via increased matrix synthesis and for contraction of the tissue. In many cases this expansion of the extracellular matrix impedes normal function of the organ. For this reason much interest has centered on the derivation of myofibroblasts and the factors that influence their differentiation, proliferation, extracellular matrix synthesis and their survival. Further understanding of how fibroblast differentiation and myofibroblast phenotype is controlled may provide valuable insights into future therapies that can control fibrosis and scarring.

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I. Introduction

Fibroblasts are present in many tissues in the body, normally in a relatively quiescent state and are mainly responsible for the production and turnover of extracellular matrix (ECM) molecules. It is now approximately 35 years since it was first reported that fibroblasts are also capable of changing during tissue repair processes to a contractile phenotype which is involved both in increased extracellular matrix production and contraction during the repair process. Since that time, much interest has centered on the control of this cell and in particular the factors that control fibroblast phenotype, proliferation, extracellular matrix production and their disappearance as tissue repair resolves. It is now apparent that the fibroblast and the differentiated cell it gives rise to, the myofibroblast, is involved in tissue repair or the response to injury in many tissues in the body and is also an important cell in numerous pathological settings, in particular pathological fibrosis and scarring in a number of organs and in the stromal response around tumours. In this review we will discuss the various roles of the myofibroblast, its derivation, control of its phenotype and its disappearance during scar resolution.

II. Inflammation and Wound Healing

A. Inflammation

Tissue repair in all organs begins with inflammation which represents the defining biological response to trauma in adults. Inflammatory reactions are triggered by a diverse range of events including, amongst others, physical injury, infection and exposure to toxins. In the case of physical trauma, platelet aggregation forms a hemostatic plug and blood coagulation forms the provisional matrix. Platelets release growth factors and adhesive proteins that stimulate the inflammatory response and induce cell migration via chemotaxis into the wound environment. Likewise, in immunological and toxic injuries, the immune response triggers the recruitment of inflammatory cells.

Important cells involved in the process include haematogenous cells, and associated resident macrophages and lymphocytes.

In the early stages of acute inflammation, polymorphonuclear granulocytes (polymorphs) are the predominant infiltrating leucocytes. If the
tissue is infected by pus producing bacteria, there is sustained and enhanced polymorph infiltration. If the bacteria survive long enough, abscess formation may result. The resulting pus consists of polymorphs, cell debris and exudate (Ryan and Majno 1977).

Subsequently the intensity of the inflammatory response subsides and the polymorph infiltration is replaced by monocyte infiltration. Polymorphonuclear cells are lost during this phase by apoptosis. When the monocyte reaches the extravascular tissue it undergoes transformation into the larger phagocytic macrophage which is then involved in removal of debris. Following activation, macrophages also secrete a wide range of biologically active mediators which are involved in tissue destruction (proteases, oxygen derived free radicals), chemotaxis (cytokines, chemokines), vascular haemodynamics (thromboxane A₂, prostaglandins) and fibrogenesis (growth factors, “remodelling” matrix metalloproteinases [MMPs] including collagenases and elastase) (Cotran et al. 1989). There is however heterogeneity amongst macrophage populations, with different stimuli producing different activation states. Kupffer cells in the liver are resident macrophages that have a similar role in liver inflammation through release of reactive oxygen species and cytokines (Bataller and Brenner 2005). In chronic inflammation, macrophage accumulation persists with their numbers supplemented by local proliferation (Ren et al. 1991) and re-circulation (Lan et al. 1993).

B. Wound Healing

Wound healing is the universal response to the inflammation that follows injury. It consists of a series of consecutive but overlapping events: these include cell proliferation, migration, extracellular matrix deposition (collectively known as fibrogenesis), resolution and remodeling (Fig 1).

The provisional fibrin-fibronectin matrix acts as a scaffold for the cell adhesion and migration. In cutaneous wounds, keratinocytes start migrating to fill the wound defect within hours. Local proliferation of these cells is important in larger wounds where migration of cells alone is insufficient to close the defect. In addition, keratinocytes are a major source of growth factors which stimulate fibrogenesis and angiogenesis in the tissue below; these include
transforming growth factor-beta (TGFβ), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and keratinocyte growth factor (KGF). The induction of these factors is driven at least in part by factors such as the hypoxia caused by the loss of vascular perfusion in this area, and subsequent induction of hypoxia inducible transcription factors. Fibroblasts quickly move into the provisional matrix, and are the principal basis of fibrogenesis. Their rapid proliferation is an important early event in response to injury, mitogenesis exponentially increasing at the site of injury within days in a renal model of injury (Hewitson et al. 1995) and also in skin wounds. Fibroblasts are the main source of the ECM proteins, mostly collagen and fibronectin, that constitutes the newly formed granulation tissue thereby providing structural integrity to the wound, while specialised fibroblasts, so-called myofibroblasts, provide the force for wound contraction, an efficient and important part of wound closure. Importantly, fibrogenesis continues as long as these cells persist in the wound; their removal or loss by apoptosis being part of the transition between granulation tissue and healing or scar formation (Darby et al. 1990; Desmouliere et al. 1995).

Providing adequate blood supply to the newly formed granulation tissue is an important part of healing. Again a number of pro-angiogenic growth factors are released at the site of injury, in an attempt to provide renewed vascularisation (Li et al. 2003). These include transforming growth factor-beta-1 (TGFβ1), basic fibroblast growth factor (FGF-2), angiopoietin, platelet derived growth factor (PDGF) and VEGF and directly and indirectly contribute to revascularisation by promoting the formation of new capillaries and stimulating proliferation of endothelial cells, migration and tube formation (organization) through the growth factor receptors expressed on endothelial cells and pericytes.

Finally, it is now accepted that the matrix is subject to remodelling by collagenous and non-collagenous proteinases, including metalloproteinases and plasmin/plasminogen family. Although the functional significance of these proteases is poorly understood it is accepted that the balance between collagen synthesis and degradation is an important factor in determining the extent of ECM accumulation (Yaguchi et al. 1998; Li et al. 2003).
While the type and size and depth of injury are important, scalpel incisions causing less injury than burns or excisional wounds for instance, the body’s response is surprisingly consistent, not only for the different types of trauma but also the response in different organs in general. The response of internal organs to parenchymal injury is therefore much the same as that which occurs in skin wound healing.

C. Healing vs Scarring

The wound healing response is an attempt to repair injury and restore tissue function. However, whereas acute wounds go through this linear series of events, chronic non-healing wounds do not. Some areas of chronic wounds are in different phases at the same time, and progression to the next phase does not occur in the same co-ordinated manner. The end result of this is the failure of wound healing and accumulation of excess matrix, so-called scarring. The most obvious example of this is in the skin in hypertrophic and keloid scarring where healing fails to resolve. However, internal organs also show analogous scarring with the liver, lung and kidney being obvious examples where pathologies induce fibrosis and scarring that may or may not resolve over time.

D. Distinction between Healing and Scarring

Although the biology of healing and scarring remain similar, ultimately it is the extent and nature of inflammation that distinguishes the two processes.

While this process in many respects resembles a recapitulation of embryogenesis, there is an important distinction. Fetuses may heal and do not scar or show only limited scarring. Fetal wound healing therefore represents the pinnacle of wound biology - restoration of tissue function without the formation of significant amounts of scar tissue. Conversely, complete regeneration of complex tissue and organs in adults is usually precluded by fibrotic reactions that lead to scarring (Harty et al. 2003). The distinction between wound healing in the fetus and the adult has not surprisingly been the subject of much attention.
Originally it was thought the sterile environment of the amniotic fluid accounted for the observed difference in fetal healing. However the observation that marsupials heal without scarring in the mother's pouch suggested otherwise. Ultimately it has been a serendipitous finding in the immunologically abnormal MRL mouse that has provided the greatest insight (Heber-Katz et al. 2004). MRL mice have a major defect in immune regulation, which results in a number of autoimmune defects. When ear hole punches are used to identify mice in laboratory colonies, the holes heal without scarring with a regeneration of the cartilage that forms the structural basis of the ear. Furthermore these exciting findings have been duplicated in a model of cardiac fibrosis (Leferovich et al. 2001), suggesting that inflammation, or perhaps more correctly, the nature of the inflammation determines the balance between restoration, healing and scarring. Other tissues may show apparently reduced scarring with the oral cavity being an obvious example. It has been suggested that local fibroblasts in the oral cavity behave more like fetal fibroblasts in showing less contractile properties and greater motility, though this is somewhat controversial (Shannon et al. 2006).

E. Cellular Basis of Healing and Scarring

Important cells involved in the process include haematogenous cells, and connective tissue cells such as fibroblasts and associated resident macrophages and lymphocytes.

Monoclonal antibodies have been used by a number of groups to phenotype the infiltrating haematogenous cells in various forms of inflammation. In renal disease (Alexopoulos et al. 1989; Hooke et al. 1987; Sabadini et al. 1988), the interstitial infiltrate is similar in nearly all forms of tubulointerstitial injury secondary to glomerulonephritis and resembles that seen in primary tubulointerstitial nephropathies and allograft rejection (Cameron 1992). T lymphocytes form the majority of haematogenous cells present, with monocytes, B cells, natural killer and plasma cells making up the remainder. Within the T-lymphocyte population there is variability in the relative proportion of T-cell subsets (Cameron 1992). Patients with idiopathic
pulmonary fibrosis generally demonstrate a neutrophil predominant alveolitis. Likewise in the lung, the specific pattern of T-lymphocytes is related to the etiology. Increased CD4+ lymphocytes are often found in the alveolar space in patients with pulmonary sarcoidosis while individuals with hypersensitivity pneumonitis may have increased numbers of CD8+ cells. (Paine and Ward 1999).

The paucity of specific markers for fibroblasts however has meant that their enumeration has been largely neglected. The fibroblast is however central to both wound healing and the pathogenesis of organ fibrosis. For instance, selective deletion of fibroblasts by transfection with a gene that results in cell death, herpes virus thymidine kinase, is sufficient to prevent renal interstitial fibrosis after injury (Iwano et al. 2001).

III. Fibroblasts and Myofibroblasts

A. Fibroblasts

The fibroblasts present in various connective tissues represent a heterogeneous population of cells. Other than the myofibroblast, first described by Gabbiani (Gabbiani et al. 1971); (Majno et al. 1971), there is no formal nomenclature to define fibroblast sub-phenotypes (Trelstad and Birk 1985). An important question is therefore, what is a fibroblast?

Ultrastructurally, fibroblasts are identified on the basis of their stellate appearance with elongated, branching processes (Takahashi-Iwanaga 1994). They have prominent rough endoplasmic reticulum and Golgi apparatus, characteristic of cells with a high biosynthetic activity (Sappino et al. 1990). Even though fibroblasts have been extensively studied in vitro, information concerning their in vivo differentiation is limited (Sappino et al. 1990). Several groups have attempted to identify fibroblasts on the basis of immunohistochemical staining, with limited success (see Table I). These attempts have been largely unsuccessful due to the poor antigenicity of fibroblasts and the non-specific nature of the antigen used. Many of the commonly used antibodies for macrophages for instance overlap in specificity with fibroblast markers (Inoue et al. 2005) and markers based on the fibroblast role in extracellular matrix (collagen) production and assembly such
as prolyl hydroxylase are only partially specific since other cell types can deposit extracellular matrix molecules and stain for this marker. The isolation of the fibroblast specific protein, FSP-1, and generation of antisera to it, has therefore been a substantial step in elucidating the role of renal fibroblasts (Strutz et al. 1995), at least in the mouse.

B. Myofibroblasts

1. Myofibroblasts in Tissue Repair

Early in normal tissue repair the fibroblasts that invade the wound resemble active but undifferentiated fibroblasts with abundant rough endoplasmic reticulum, as would be expected for extracellular matrix producing cells (Darby et al. 1990). As healing and the repair process progresses, myofibroblasts begin to appear (Figures 2 and 3). Myofibroblasts are mesenchymal cells with features of both fibroblasts and smooth muscle cells (Sappino et al. 1990). Ultrastructurally these cells are characterised by features of both fibroblasts; including spindle shape, prominent cytoplasmic projections, abundant rough endoplasmic reticulum, and features of smooth muscle cells; -longitudinal cytoplasmic bundles of microfilaments and multiple nuclear membrane folds (Sappino et al. 1990). Myofibroblasts also display a “fibronexus” which connects the intracellular microfilaments both to other myofibroblasts and surrounding extracellular matrix (Eyden 1993). The fibronexus may thus mediate continuity between contractile filaments and matrix proteins (Eyden 1993). Expression of the protein smoothelin may reliably distinguish smooth muscle cells from myofibroblasts, with a number of reports suggesting that smoothelin is exclusively found in smooth muscle cells (Miller and Marshall 1980; van der Loop et al. 1996).

2. Differentiation Markers

Cytoskeletal protein differentiation markers have been used as a marker of mesenchymal phenotype (Sappino et al. 1990). Microfilaments, intermediate filaments and microtubules are the 3 components of the eukaryotic cell cytoskeleton. Microfilaments consist mainly of actin,
intermediate filaments are usually a mixture of vimentin and/or desmin, while the microtubules are predominantly tubulin (Desmouliere and Gabbiani 1992).

Expression of the smooth muscle specific protein $\alpha$-smooth muscle actin ($\alpha$-SMA) is often used as a marker of myofibroblast phenotype. $\alpha$-SMA is one of six different actin isoforms (Vandekerckhove and Weber 1978) which are present in all eukaryotic cells. $\beta$ and $\gamma$ actins are present in all cells, while the distribution of $\alpha$-cardiac, $\alpha$-skeletal and $\alpha$-smooth muscle isoforms are tissue specific (Desmouliere and Gabbiani 1992).

Myofibroblasts may however differ in the smooth muscle proteins expressed. While expression of the cytoskeletal protein $\alpha$-SMA is used as an almost universal marker of myofibroblast phenotype, the same cannot be said for desmin and smooth muscle myosin. Four different myofibroblast phenotypes have been identified in skin wounds, based on morphology and the distribution of tissue specific cytoskeletal proteins. Type V myofibroblasts express vimentin only, type VA express vimentin and $\alpha$-SMA, VAD are positive for vimentin, $\alpha$-SMA and desmin, while VD are positive for vimentin and desmin only (Skalli et al. 1989); (Sappino et al. 1990); (Gabbiani 1992). Desmin containing myofibroblasts are usually only found in hypertrophic scars and fibromatoses (Skalli et al. 1989). To date myofibroblast expression of smooth muscle myosin has not been described (Gabbiani 1992).

3. Myofibroblasts in Organ Fibrosis

Myofibroblasts have been observed in practically all fibrotic conditions involving retraction and reorganisation of connective tissue and have been the subject of several reviews (Sappino et al. 1990),(Schmitt-Graff et al. 1994), (Desmouliere et al. 1992; Desmouliere et al. 2003). In addition to their involvement in skin wound healing (Gabbiani et al. 1971), (Majno et al. 1971), (Hebda et al. 1993), myofibroblasts are most commonly found, and well characterised in pulmonary fibrosis (Kuhn and McDonald 1991) (Vyalov et al. 1993), hepatic fibrosis (Schmitt-Graff et al. 1993), cardiac fibrosis (Leslie et al. 1991), and a number of carcinomas associated with an inflammatory response (Takahashi et al. 1994) such as breast (Ronnov-Jessen and Petersen 1993), pancreas and bowel (Hewitt et al. 1993); (Takahashi et al.
The role of myofibroblasts has been extensively studied in skin wound healing where they are thought to be responsible for wound contraction (Majno et al. 1971; Grinnell 1994). Studies quantifying myofibroblasts in granulation tissue have shown that the number of myofibroblasts is proportional to the rate of wound contraction (Rungger-Brandle and Gabbiani 1983). Nagle et al described interstitial fibroblasts with smooth muscle characteristics in a rabbit model of renal obstruction some 30 years ago (Nagle et al. 1973). More recently, we and others have described interstitial cells with the ultrastructural features of myofibroblasts in renal biopsies with various nephropathies (Alpers et al. 1994; Hewitson and Becker 1995; Goumenos et al. 1994; Essawy et al. 1997; Vangelista et al. 1989; Pedagogos et al. 1997). Spindle shaped interstitial cells in the fibrotic interstitium, which stain for the smooth muscle specific actin isoform (α-smooth muscle actin) (Figure 4 and 5) are thus well studied in both experimental models of disease and in human disease, consistent with their important role in scar formation.

4. Cancer Associated Stromal Myofibroblasts

Another area of research which has gained momentum in recent years is the presence of myofibroblasts in the stroma around tumours and their role in tumour progression. It is now established that myofibroblasts are present in the stroma around a number of different cancers and that these may play an important role in signalling the tumour cells, both to increase tumour progression and to promote tumour invasion (Kalluri and Zeisberg 2006). Local fibroblasts in the connective tissue again likely give rise to many of the myofibroblasts present in the stromal reaction, however, it has also been found that bone-marrow derived myofibroblasts are present (Ishii et al. 2003; Direkze et al. 2003). The presence of myofibroblasts in the tumour stroma may provide a source of growth factors which signal tumour cells, but also could be involved in connective tissue remodelling allowing expansion and invasion, indeed many myofibroblasts have been found to be present at the front of invasion in malignant tumours (De Wever and Mareel 2003). It has also been suggested that the tumour cells may induce changes in the
myofibroblast population in the stroma by paracrine mechanisms, resulting in selection for a subpopulation of highly proliferative myofibroblasts that lack p53, the cell cycle checkpoint control (Hill et al. 2005).

C. Origin of the Fibroblast

It has been assumed that a pluripotential mesenchymal stem cells can give rise to various differentiated mesenchymal cells including smooth muscle cells, pericytes and fibroblasts (Jimenez and Martinez 1992).

For many years it has been postulated that the fibroblast cell system, at least in vitro, is a terminally differentiating cell system. In studies using skin fibroblasts, Bayreuther et al. proposed that fibroblasts differentiate along a terminal lineage in the same way as haemopoietic cells (Bayreuther et al. 1988); (Bayreuther et al. 1991). Similarly in studies using rabbit renal fibroblasts Muller et al. identified 3 mitotically active progenitor fibroblasts (cell types MF I, MF II, MF III) and 3 postmitotic fibroblast cell types (PMF IV, PMF V and PMF VI) (Müller et al. 1992); (Rodemann et al. 1991). Cell type PMF VI has been shown by biochemical analysis to have the highest biosynthetic activity for various fibroblast specific components and secreted proteins (i.e. collagen and other ECM proteins) (Müller et al. 1992).

More recently however, we recognise the plasticity of cell phenotypes. Fibroblasts may be recruited from resident cell populations, circulating precursors and transformation of epithelial cells.

1. Resident Fibroblasts

Electron microscopy studies have consistently shown that in many organs there are resident populations of fibroblast-like cells that proliferate rapidly in response to injury. Local proliferation and migration from adjacent tissues, in particular the perivascular region, have generally been accepted as the mechanisms by which tissue fibroblast numbers may increase (Wiggins et al. 1993). In skin wounds, strong staining near the wound margins for proliferation markers such as bromodeoxyuridine (BrdU) (Figure 6) and for MMP-13 (collagenase) (Figure 7) is suggestive of fibroblast proliferation and
migration from surrounding unwounded connective tissue (Darby et al. 1997; Desmoulière et al. 2003).

2. Circulating Precursors

Bucala et al (Bucala et al. 1994) were able to isolate a population of so-called fibrocytes, cells in healing skin wounds that expressed both the hematogenous cell marker CD34+ and procollagen I. In circumstances of sex mismatched bone marrow transplants, they were able to show mismatched DNA in these cells, confirming that they were of donor origin. Likewise, there are now also reports that bone marrow derived fibrocytes traffic to areas of renal (Grimm et al. 2001) and pulmonary (Phillips et al. 2004; Schmidt et al. 2003) fibrosis.

3. Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition, so-called EMT, is the process that facilitates the derivation of a multitude of functionally specialized cells, tissues and organs in the developing embryo (Khew-Goodall and Wadham 2005). Consistent with the recapitulation of developmental programs during “wound healing”, a reverse process occurs in response to tissue injury, where epithelial cells acquire features of mesenchymal cells. During EMT, epithelial cells lose polarity and cell-cell contacts and undergo dramatic cytoskeletal remodelling. Concurrent with the loss of epithelial cell adhesion and cytoskeletal components, cells undergoing EMT acquire mesenchymal cell expression profiles and migratory phenotype. EMT is therefore in essence, a process of reverse embryogenesis.

Renal tubular EMT, by definition, is a process in which renal tubular cells lose their epithelial phenotype and acquire features characteristic of mesenchymal cells (Liu 2004a). The process of EMT has been found to be particularly significant in the pathogenesis of tubulointerstitial fibrosis that accompanies all progressive renal disease (Liu 2004a). Pioneering work from Strutz et al (Strutz et al. 1995) indicated that renal tubular epithelial cells can express fibroblast markers in disease states, suggesting that epithelial to mesenchymal transition is a potential source of cells in pathological kidneys.
Likewise TGFβ1 can induce alveolar EMT in human lung epithelia cells; a process inhibited by smad2 gene silencing, but not by MEK inhibitors (Kasai et al. 2005).

Elegant renal experiments in a murine model of unilateral ureteric obstruction have attempted to address the relative importance of the various potential sources of fibroblasts (Iwano et al. 2002). Using bone marrow chimeras and transgenic reporter mice, the authors were able to estimate that resident fibroblasts, EMT and circulating precursors contributed 52%, 38% and 9% of the fibroblast burden respectively (Iwano et al. 2002). In the kidney, it remains unclear if myofibroblasts can be derived directly from EMT, or only indirectly via a prior transition into fibroblasts.

D. Origin of the Myofibroblast

The derivation of the myofibroblast phenotype is also complex. Its close association with fibroblasts in parenchymal wound healing systems (Clark 1989) suggests that it may modulate from fibroblasts. Much of the rationale for this hypothesis is derived from in vitro studies where quiescent fibroblasts express α-smooth muscle actin upon stimulation (Ronnov-Jessen, L. and Petersen 1993).

Ultrastructural studies in sequential skin wounds also suggest that myofibroblasts are derived from resting fibroblasts in the wound margin (Bouissou et al. 1988, Darby et al. 1990). Bayreuther et al. believe that the myofibroblast corresponds to the post-mitotic fibroblast PMF IV (Bayreuther et al. 1991). However, other possibilities include a pericyte origin (Nehls and Drenckhahn 1993) and even a macrophage derivation has been postulated (Bhawan and Majno 1989). At least in some specialised fibrogenic systems, myofibroblast-like cells may be derived from a specialised cell type, as in renal glomerulosclerosis where myofibroblasts represent activated mesangial cells (Alpers et al. 1992). Likewise in hepatic fibrosis, myofibroblasts are derived from both vitamin A storing hepatic stellate cells and activated peri-sinusoidal cells (Hines et al. 1993). The relative importance of these two cell
type may depend on the origin of the liver injury (Bataller and Brenner 2005). In experimental models of hepatic fibrosis induced by carbon tetrachloride it is mainly the hepatic stellate cells that are activated and subsequently differentiate into myofibroblasts, while in models using cholestasis (bile duct ligation) the perisinusoidal and connective tissue fibroblasts present in portal regions become activated and participate in the fibrotic response, expressing myofibroblast markers in the process.

IV. Fibrogenic mediators

A. Fibrogenic Mediators

The activity of fibroblasts and their subsequent differentiation into myofibroblasts is dependent on a combination of the action of growth factors and other soluble mediators, extracellular matrix components and mechanical stress.

1. Pro-fibrotic Cytokines and Growth Factors

A long list of cytokines (including polypeptide growth factors) are likely to play a role in fibrogenesis. Growth factors and other cytokines may be produced by both resident and infiltrating inflammatory cells. A hierarchy is likely to exist amongst the many cytokines considered to be putative mediators of injury (Atkins 1995).

The macrophage is a central figure in chronic inflammation because of the great number of biologically active products it can produce (Vangelista et al. 1991). Growth factors the macrophage is known to synthesise and secrete include PDGF (Zhuo et al. 1998), FGF-2 (Baird et al. 1985) and TGFβ1 (Associan et al. 1987). Compelling evidence for the role of macrophages as mediators of injury has been provided by renal studies in which macrophage infiltration was reduced by X-ray irradiation (Diamond and Pesek-Diamond 1991) and anti-macrophage serum (Matsumoto and Hatane 1989). Such treatments resulted in a concomitant decrease in severity of injury. Although these studies establish a role for macrophage mediated injury they do not specifically establish a direct role in up-regulation of fibrogenesis.
Importantly fibroblasts express receptors for a number of cytokines including PDGF (Alpers et al. 1993); (Alpers et al. 1994), TGFβ1 (Noronha et al. 1995) and tumour necrosis factor-α (TNFα) (Noronha et al. 1995). These factors may mediate their recruitment and activation during injury. Alvarez et al. described heterogeneity in the paracrine response of fibroblasts derived from different anatomical locations, once again highlighting the heterogeneous nature of the fibroblast phenotype (Alvarez et al. 1992).

Although primarily thought to be a producer of extracellular matrix, fibroblasts are also capable of producing prostaglandins (Korn 1985), erythropoietin (Bachmann et al. 1993) and cytokines (Noronha et al. 1995). Fibroblasts are therefore capable of both autocrine and paracrine responses, further amplifying the fibrogenic response.

Several authors have now consistently shown that the acquisition of myofibroblast features in skin (Desmoulière et al. 1993), renal (Masterson et al. 2004) and lung fibroblasts (Evans et al. 2003) is TGFβ1 mediated. A role for TGFβ1 in fibrogenesis has been hypothesised on the basis of observations that TGFβ1 neutralising antibodies (Border et al. 1990) and the natural TGFβ1 binding glycoprotein, decorin (Border et al. 1992) abrogate renal fibrogenesis.

In some cases, TGFβ1 activities are mediated by downstream activation of connective tissue growth factor (CTGF). An immediate early response gene, CTGF is a potent stimulus for myofibroblast differentiation and matrix synthesis in a variety of tissues (Leask and Abraham 2004). Over expression of CTGF is a consistent feature of fibrotic lesions; and the combination of TGFβ1 and CTGF promotes ongoing fibrosis (Leask and Abraham 2004).

Local subcutaneous application of granulocyte-macrophage colony stimulating factor (GM-CSF), a growth factor known for its hemopoietic effects, induces the accumulation of myofibroblasts (Rubbia-Brandt et al. 1991) though it has been postulated that this occurs through macrophage activation and subsequent secretion of macrophage-derived factors such as TGFβ1 since GM-CSF applied directly to fibroblasts in vitro has no effect on α-SMA expression.

Cytokines and growth factors act in a synergistic paracrine fashion to stimulate tissue formation (Clark 1989). While the effect of these agonists on
the quantity of collagen produced has been studied extensively by cell culture methods, results are difficult to apply to in vivo situations because of complicated fibroblast inflammatory cell and cytokine interactions (Larjava et al. 1990).

2. Other Soluble Pro-fibrotic Factors

A variety of mesenchymal cells, including fibroblasts, express receptors for the vasoactive mediators endothelin-1 and angiotensin II. Both have pro-fibrotic effects in cultured gingival fibroblasts (Ohuchi et al. 2002).

3. Extracellular Matrix

ECM is not only generated by fibroblasts but also acts as a potent regulator of fibroblast function. Changes in the composition of ECM can therefore directly stimulate fibrogenesis.

In most cases these effects are mediated through interaction with integrins (Paine and Ward 1999; Geiger et al. 2001). Both fibroblast proliferation and collagen synthesis are influenced by adhesion to extracellular matrix. The more firmly that lung fibroblasts are anchored, the more they proliferate and synthesize collagen (Paine and Ward 1999). However, this does not seem to hold true for fibroblasts isolated from fibrotic lungs, which continue to proliferate on a soft substrate, in an anchorage-independent manner (Torry et al. 1994), suggesting that fibroblast responses to ECM signals are critical components that restrict fibroblast activity in the normal homeostasis (Paine and Ward 1999).

Cytokine effects may also be enhanced by the ability of ECM to bind soluble mediators. For example collagen IV and chondroitin sulphate proteoglycan binds TGFβ1, while heparan sulfate proteoglycan binds FGF-2 and TGFβ1 (Schlondorff 1993). ECM may thus act as a so called "reservoir" of growth factors (Schlondorff 1993; Li et al. 2003).

Nonenzymatic glycation is a ubiquitous reaction between reducing sugars and polypeptides that ultimately generates irreversibly advanced glycated end products (AGEs). This occurs during normal aging but to a greater degree in diabetes, where interaction between AGEs and their binding proteins lead to
the activation of a range of cytokines. Specifically, AGEs have been shown to promote EMT (Oldfield et al. 2001), ostensibly through generation of TGFβ1.

Fibronectin is a multifunctional protein found as both a soluble plasma form and insoluble constituent of ECM, with a number of splice variants further contributing to fibronectin polymorphism. The human EDA variant (and EIIIA homologue in rats) is abundantly expressed during embryogenesis and wound healing/fibrosis where its expression closely parallels αSMA expression. Molecular studies have shown that the ED-A isoform is a critical co-factor required for TGFβ1 induction of myofibroblast differentiation (Serini et al. 1999).

4. Mechanical Tension

Mechanical stress is an important mediator of fibroblast differentiation in those of tissues where mechanical tension and transmission of force occurs, such as the skin. It is thought that the presence of ED-A fibronectin splice variant, mechanical tension and TGFβ1 are required to obtain the full differentiation to the myofibroblast phenotype (Tomasek et al. 2002), while the presence of mechanical tension may on its own produce a partial phenotype where cells possess stress fibres but lack α-smooth muscle actin positive immunostaining. Recent studies have shown that externally applied mechanical loads can lead to rapid induction of changes in the extracellular matrix composition, suggesting adaptation of the matrix to mechanical loading (Chiquet et al. 2003) and a putative stretch responsive promoter has been found in genes which respond to mechanical tension or stretch, such as tenascin and collagen XII (Chiquet 1999). Signalling through this pathway may also account for the differences observed between stressed fibroblast populated collagen lattices and those that are free floating in vitro. Floating collagen lattices are remodelled and 'contract' over time, while stressed lattices express greater levels of α-smooth muscle actin and are thus more capable of true contraction.
5. Naturally Occurring Inhibitors of Fibrogenesis

Increasingly we recognise that not only are fibroblasts regulated by a number of pro-fibrotic factors, but also that there are a number of endogenous factors that down-regulate fibroblast function. The most studied of these, hepatocyte growth factor (HGF) (Liu 2004b), bone morphogenic factor-7 (BMP-7) (Zeisberg et al. 2004) and the hormone relaxin (Samuel and Hewitson 2006), maintain tissue integrity by antagonising TGFβ1 activity and signaling. After injury, reduced levels of these endogenous factors can accelerate fibrosis (Eddy 2005).

B. Signal Transduction in Fibroblasts

The microenvironment following tissue injury therefore represents a complex mixture of inflammatory, fibrogenic and anti-fibrogenic mediators. How do fibroblasts recognise and make sense of what are often conflicting signals?

As with other inflammatory circumstances in general, growth factors and cytokines signal through the numerous well described signal transduction pathways.

1. Receptor Binding

Receptors acting at the membrane level transduce extracellular signals into a cytosolic one, where subsequent communication of signals involves translocation of intracellular signaling components.

These membrane receptors take various forms. Arguably the most important fibrogenic factors, TGFβ1 and PDGF, signal through a receptor serine/threonine kinase and tyrosine kinase respectively.

TGFβ1 belongs to a superfamily of structurally related regulatory proteins which include three mammalian TGF isoforms (TGFβ1 -β2, -β3), activin/inhibin and bone morphogenic proteins. Signaling through functional cooperation of the Smad proteins, the TGF-β receptor phosphorylates receptor-regulated Smads (Smad2, Smad3), a pathway that is strictly
regulated at various levels, including inhibition by Smad7 which complexes with Smad2 and 3 to prevent phosphorylation (Schnaper et al. 2002).

PDGF is secreted as a homodimer, composed of two A-, B-, C- or D-chains or a heterodimer composed of an A- and B-chain which complex variously with α or β receptor subunits. The PDGF B chain, as part of either PDGF-BB or PDGF-AB, is a potent mesenchymal cell mitogen (Floege 2002), while various PDGF-BB isoforms mediate chemotaxis, contraction and the activity of other factors (Johnson et al. 1993). Induction of PDGF-B type receptors on smooth muscle cells is seen in atherosclerosis and renal allograft rejection has long been recognised (Fellstrom et al. 1989).

Likewise the janus tyrosine kinase family is important for cytokine signaling, while G-protein transmembrane receptors signal through associated effector enzymes such as Ras GTPases, a convergent point in signal cascades for many mitogens (Khwaja et al. 2000).

Finally, Integrins have a particularly important role in fibrogenesis, mediating the interaction between mesenchymal cells and ECM. Expressed on both leukocytes and parenchymal cells, they bind to extracellular matrix proteins and, in the case of leukocytes, to ligands on other cells. Integrin proteins are heterodimers that consist of paired α and β chains. At least 14 α subunits and eight β subunits have been characterized thus far, with the pairing of a subunits determined specificity (Paine and Ward 1999). Integrins containing the β1 chain or the αv chain are particularly important for their role in binding to matrix proteins. The multiple integrin heterodimers allow fine control both of the matrix components to which the cell can adhere, and therefore the signals transduced (Geiger et al. 2001). In many cases effects are mediated by activation of focal adhesion kinase, a member of the tyrosine kinase family (Yasuda et al. 2002).

2. Non-receptor Mediated Signaling

In addition to receptor signal transduction, non-receptor mediated signaling, for example by nitric oxide, is an important means of influencing fibroblast function (Hewitson et al. 2002).
3. Second messenger pathways

The activation and translocation of kinases from the cytoplasm to the nucleus provide the link between extracellular signals and gene expression. Again these pathways take many forms and include, amongst others, the mitogen activated protein kinase, phosphatidylinositol 3-kinase and protein kinase C cascades. In each case the end result is the phosphorylation and activation of nuclear transcription factors, with gene transcription being controlled by the interaction of transcription factors with specific DNA sequences known as promoter or enhancer motifs (Mann and Smart 2002).

Various transcription factor families have established roles in fibrosis. Nuclear factor-κB (NF-κB), perhaps the best known of the nuclear transcription families, promotes the upregulation of cytokines involved in inflammation (Guijarro and Egido 2001). Mesenchymal cell differentiation is under the control of NF-κB (Klahr and Morrissey 2000) and MyoD, a member of the basic helix-loop-helix family (Mayer and Leinwand 1997), while various members of the Kruppel-like transcription factor family regulate collagen transcription in hepatic stellate cells (Mann and Smart 2002). Conversely, peroxisome proliferator activated receptor γ (PPARγ) is active in quiescent mesenchymal cells (Mann and Smart 2002), suggesting that it may block fibrogenesis.

C. Fibroblast Responses

The fibroblast response to this microenvironment includes differentiation, migration, proliferation and increased ECM synthetic activity.

1. Differentiation

Molecular regulation of (myo)fibroblast differentiation remains poorly understood. TGFβ1 seem to play a central role, having been shown consistently to upregulate αSMA expression in a diverse range of fibroblasts (Desmoulière et al. 1993; Masterson et al. 2004; Evans et al. 2003). However, although the importance of TGFβ1 is well recognized, TGFβ1 upregulates only a subset of smooth muscle differentiation markers in non-smooth muscle
cells (Hautmann et al. 1999). How these override the repressor activity that normally suppresses expression of αSMA is unclear (Hautmann et al. 1999).

In fibrocytes, the TGFβ1 induced increases in αSMA expression are paralleled by a loss of CD34 expression (Abe et al. 2001; Schmidt et al. 2003).

The ED-A isoform of fibronectin is required for TGFβ1 mediated acquisition of myofibroblast features by human subcutaneous fibroblasts (Serini et al. 1999). The collagenase MMP-2 is necessary and sufficient to stimulate myofibroblast transition (Cheng and Lovett 2003).

Renal mesangial cells isolated from C/EBPδ null mice express much less αSMA than their wild-type counterparts, suggesting the involvement of the transcription factor C/EBPδ in myofibroblast differentiation (Takeji et al. 2004).

A more recent regulator of myofibroblast differentiation that may be involved in phenotypic change from fibroblast to myofibroblast is the clotting enzyme thrombin. Thrombin has, in addition to its clotting activity, a cell surface receptor that is activated by cleavage of the receptor N-terminus (extracellular domain). Proteinase-activated receptors exist which are activated by thrombin cleavage; specifically proteinase-activated receptor-1 (PAR-1). PAR-1 activation has been shown to stimulate myofibroblast differentiation in lung fibroblasts (Bogatkevich et al. 2001) and in renal fibroblasts, at least in vitro (Hewitson et al. 2005).

2. Migration

Leucocytes and mesenchymal cells such as fibroblasts, are attracted to the site of injury by various chemotactic factors including TGF-β. Indeed TGF-β is chemotactic for monocytes and fibroblasts; fibroblast chemotaxis being signalled through Smad3. Interestingly TGF-β stimulation of the myofibroblast phenotype in fibroblasts is not dependent on Smad3 (Flanders et al. 2003). In many cases these cells are in turn activated to produce further proinflammatory cytokines, thus initiating a cascade of events that can lead to fibrosis (Kovacs and DiPietro 1994).

Migration is dependent upon interaction between cells and surrounding ECM. For fibroblasts to move from place to place, cells must form and release
the adjacent ECM. Several studies indicate that the profile of integrin expression in motile fibroblasts differs from that in resting fibroblasts (Paine and Ward 1999; Clark 1993). Fibroblasts therefore display a phenotypic switch in their integrin expression, which may in part explain the temporal distinction in the various phases of wound healing.

In a rabbit model of crescentic glomerulonephritis, Wiggins et al. (Wiggins et al. 1993) demonstrated that fibroblast-like cells migrated from a perivascular location into the interstitium, accumulating in a periglomerular distribution. Such migration may be in response to chemotactic substances produced by the inflamed glomerulus (Wiggins et al. 1993). Very similar findings have been found in a combined model of periarterial fibrosis and proliferative glomerulonephritis (Faulkner et al. 2005).

There is evidence to suggest that activated fibroblasts also secrete stromelysin and collagenases which assist with their interstitial migration by dissolving the surrounding interstitial matrix (Kuncio et al. 1991).

3. Mitogenesis

Fibroblasts derived from fibrotic lesions continue to exhibit a hyperproliferative growth pattern when compared with kidneys isolated from normal kidneys (Rodemann and Müller 1990).

Cytokines are mitogenic through not only direct effects but also indirectly by stimulating the release of other cytokines (Kovacs and DiPietro 1994).

The most important function of PDGF is to stimulate proliferation of mesenchymal cells including fibroblasts and smooth muscle cells. These cells not only respond to PDGF in a paracrine fashion, but also have an autocrine pathway available through peptide production (Noronha et al. 1995).

TGFβ1 may act as both an inhibitor and promoter of cell proliferation. TGFβ1 has a biphasic response on mesenchymal cells in culture, inhibiting proliferation at high concentrations, while being mitogenic in low concentrations. These paradoxical effects are thought to be the result of interaction with other growth factors (Kovacs, E. J. and DiPietro, L. A.94).

Likewise GM-CSF stimulates in-vitro proliferation of not just haemopoietic cells, but also of a number of mesenchymal cells including fibroblasts (Denhar et al. 1988).
4. Matrix Synthesis

The extracellular material once only described as reticulin is now known to consist of a complex matrix of collagen, proteoglycans and glycoproteins (Lemley and Kriz 1991). The distribution and composition of the extracellular matrix is surprisingly similar across various organs, at least before injury. The interstitium in the lung, liver and kidney is principally composed of the triple helical fibrillar collagens (type I and III), elastin, proteoglycans and fibronectin, while lung and alveolar basement membranes consist mainly of type IV and laminin (Paine and Ward 1999). Type I collagen is the major collagen in adult dermis (Kirsner and Eaglstein 1993). Fibrosis is associated with major alterations in both the type and quantity of matrix synthesis.

In advanced stages of disease the liver contains approximately 6 times more ECM than normal (Bataller and Brenner 2005). Increased synthesis of fibronectin by fibroblasts is thought to be an early event in both wound healing and fibrosis (Goyal and Wiggins 1991), with the fibronectin matrix providing a scaffold on which subsequent interstitial collagen is fixed (Goyal and Wiggins 1991); (Clark 1989). Fibronectin is involved in adhesion of cells to substrate and other cells, and as such probably represents a primer in organisation of fibrotic tissue before the appearance of collagenous protein (Hynes and Yamada 1982). Furthermore as described above, synthesis of the fibronectin splice variant EDA, and its rat orthologue EIIIA, have important pro-fibrotic effects including promoting EMT.

Our recent work in renal disease suggests that up-regulation of the various collagen isotypes is temporally distinct with increased accumulation of interstitial collagen I and IV occurring well before increases in collagen III (unpublished observations). Conversely, although Type III collagen is a minority component of normal adult skin, aberrant synthesis occurs within 48 hours of skin wound healing (Kirsner and Eaglstein 1993).

Most commonly recognised for their collagen producing ability, fibroblast-like cells are the intrinsic collagen producing cells in most organs. However, they are not the only cells capable of collagen production with for example collagen producing cells in the renal cortex known to include glomerular (Kreisberg and Karnovsky 1983); (Scheinman et al. 1992) and tubular (Creely
et al. 1988); (Scheinman et al. 1992) epithelial cells, mesangial cells (Scheinman et al. 1992) and interstitial fibroblasts (Rodemann and Müller 1991). Fibroblasts cannot be grown from isolated cortical glomeruli which suggests that they are not necessary for matrix production, at least in glomerulosclerosis (Alvarez et al. 1992).

5. Contraction

Wound contraction has long been recognised as feature of skin wound healing. Contraction was originally thought to result form the polymerisation of collagen fibrils, due therefore to the tensile strength of collagen (Botsford 1941), (Howes et al. 1929). Abercrombie et al described the dissociation of collagen formation and contraction at late stages of wound healing, suggesting that other factors may be involved (Abercrombie et al. 1954). This group was also able to demonstrate that wounds in ascorbic acid deficient animals can close independently of collagen formation (Abercrombie et al. 1956). Although these studies did not contradict the role of physiochemical contraction, they cast doubt on the notion that collagen polymerisation alone was sufficient to account for wound contraction.

The observation that fibroblasts acquire prominent cytoplasmic microfilaments therefore suggested that they perform a contractile function in wound healing (Majno et al. 1971; Gabbiani et al. 1972). Indeed, strips of granulation tissue have been shown to have the ability to contract ex vivo in response to various compounds that stimulate smooth muscle cell contraction (Higton and James 1964; Majno et al. 1971).

Not surprisingly then, studies quantifying myofibroblasts in granulation tissue have shown that the number of myofibroblasts is proportional to the rate of wound contraction (Rungger-Brandle and Gabbiani 1983). Elegant experiments from Hinz et al (Hinz et al. 2001) demonstrated that cultures of lung myofibroblasts, but not fibroblasts, are able to deform silicon sheets when used as in vitro substrates for fibroblast attachment. The myofibroblast fibronexus connects the intracellular microfilaments both to other myofibroblasts and surrounding extracellular matrix (Eyden 1993) thereby mediating contraction.
However, it is worth remembering that wound contraction may be due not only to the contractile properties of specialised mesenchymal cells but also indirectly through cell migration. Ehrlich et al propose that fibroblast locomotion (or traction) is sufficient to reorganise extracellular matrix (Ehrlich 1988). This postulate is based on the observation that collagen gel lattices populated with growing fibroblasts are able to contract in the absence of stress fiber formation. Contraction of the gel occurred during cell migration, ceasing once cells were no longer motile (Ehrlich 1988).

Contraction in the myofibroblast is mediated by $\alpha$-smooth muscle actin and by non-muscle myosin as myofibroblasts do not or only rarely express smooth muscle myosin. The pathway for contractile signalling involves Rho kinase activation (Grinnell et al. 1999) and blockers of Rho kinase inhibit PDGF stimulated lattice contraction (Abe et al. 2003; Lee et al. 2003).

V. Regulation of Fibrogenesis

A. Resolution or Progression

Resolution of inflammation represents an important step in limiting the chronicity of inflammation (Kuncio et al. 1991). Fibrogenesis continues as long as permissive conditions exist. The persistent accumulation of inflammatory cells is associated with the continued destruction of renal tissue and deposition of scar tissue. If unchecked this leads to progression of scarring and ultimately in the case of internal organs, organ failure. Abscess formation is an extreme example of uncontrolled inflammation, where disintegrating neutrophils not only degrade tissue but also amplify the immune response (Ryan and Majno 1977).

Ideally inflammation will resolve efficiently, resulting in complete restoration of tissue structure and function. This occurs frequently in skin wounding, and can occur in the renal context, as with post-streptococcus glomerulonephritis (Vogl et al. 1986) and ischaemic acute renal failure (Forbes et al. 2000). Such resolution of inflammation may ultimately be the factor that distinguishes between “healing” and “scarring”.

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In order for resolution of inflammation to occur, the following events must take place; 1) removal of stimulus, 2) dissipation of mediators, 3) cessation of cell infiltration and finally 4) clearance of inflammatory cells (Haslett 1992).

In particular, how inflammatory cells are removed remains controversial, with ex-migration, phenotypic modulation and cell death all possible mechanisms.

Ultrastructural studies in skin wound healing suggest that the prevalence of αSMA negative fibroblastic cells increases at the completion of healing, consistent with phenotypic modulation or de-differentiation of wound myofibroblasts (Darby et al. 1990). However, in vitro studies suggest that the myofibroblast is a part of a terminally differentiating cell lineage, therefore arguing against de-differentiation (Bayreuther et al. 1991).

The demonstration of lymphatic macrophage drainage and trafficking in experimental glomerulonephritis (Lan et al. 1993) implicates some role for ex-migration in resolution of tubulointerstitial inflammation.

Increasing evidence suggests that apoptosis is the major mechanism of decreasing cellularity during the various stages of “wound healing”. Apoptosis is a form of cell death which does not lead to an inflammatory response and may therefore be important in resolution of inflammation and fibrogenesis (Darby et al. 1990; Desmouliere et al. 1995; Greenhalgh 1998). It is characterised by endonuclease activation resulting in rapid DNA degradation, with engulfment of the membrane-bound cellular fragments (apoptotic bodies) without release of cell contents (including inflammatory mediators) (Lu et al. 2005). Importantly apoptosis leads to the safe removal of cells by phagocytosis, whereas in contrast, necrosis provokes tissue injury and inflammation (Lu et al. 2005; Greenhalgh 1998). Qualitative and quantitative studies in skin wound healing (Darby et al. 1990; Desmouliere et al. 1995) have repeatedly shown that fibroblastic cells are removed by apoptosis at the completion of wound healing (Figure 8).

It seems likely that regulation of myofibroblast apoptosis may be involved in both impairment of tissue repair; in cases where apoptosis is increased inappropriately or prematurely, such as that seen in skin wound healing in diabetes mellitus (Darby et al. 1997). Further evidence for this
comes from studies showing that the presence of advanced glycosylation end-products in the skin may result in fibroblast apoptosis and that this can be mimicked by treatment with the AGE-modified collagen (carboxy methyl lysine [CML]-collagen) (Alikhani et al. 2005). AGE-modified matrix in vitro can inhibit attachment spreading and proliferation and induce apoptosis of fibroblasts (Alikhani et al. 2005; Darby et al. 2006). Conversely, it has been speculated that delayed or absent induction of apoptosis in myofibroblasts may result in increased scarring, such as that seen in hypertrophic scars or keloids.

B. Remodeling

Following deposition of extracellular matrix, mechanisms exist whereby this immature matrix may be remodelled as part of scarring. These include the action of proteinases (Jones et al. 1991) and scar contraction (Rudolph et al. 1992). This serves to highlight tubulointerstitial scarring may be a product of several factors other than matrix synthesis per se.

Degradation of the ECM involves proteolytic digestion by two families of enzymes, the metalloproteinases and plasminogen activator/plasmin (Alexander and Werb 1989). The identification of collagenases and their inhibitor proteins in a number of organs indicates that ECM accumulation may be a result of either increased synthesis or decreased degradation (Yaguchi et al. 1998; Eddy 2005).

The metalloproteinase family includes a number of members with various and partly overlapping specificities (Alexander and Werb 1989). This family of proteinases is divided into three groups: collagenases which degrade interstitial collagens Type I, II and III, type IV collagenases/gelatinases which degrade basement membrane collagen and gelatins, and stromelysins which degrade a broad range of substrates including proteoglycans, laminin, gelatins and fibronectin (Martrisian 1990).

The production of these enzymes by endothelial cells, fibroblasts and macrophages can be induced by a wide variety of stimuli (Jones et al. 1991). Matrix metalloproteinases are also regulated by control of activation and the presence of specific inhibitors known as tissue inhibitors of metalloproteinases (TIMP) (Martrisian 1990). The serine protein plasmin is active in degradation
of laminin and to some extent gelatin, fibronectin and collagen type III, IV and V (Alexander and Werb 1989). Much of the degradative effect of the plasmin/plasminogen system is achieved through activation of the matrix metalloproteinases (Jones et al. 1995).

The remodelling of the matrix may play an important role in regulating the survival of myofibroblasts. In granulation tissue, the application of a skin flap over a wound results in the rapid remodelling of the granulation tissue, accomplished by an induction of apoptosis in fibroblasts and other cell types such as vascular endothelium (Garbin et al. 1996). This was subsequently shown to correlate with increased MMP expression and reduced expression of TIMP (Darby et al. 2002). In a similar vein, changes in the levels of MMPs and TIMPs has been shown in fibrotic liver to be able regulate loss of myofibroblasts in fibrotic regions, with exogenous TIMP or MMP inhibitor being able to block the induction of apoptosis (Murphy et al. 2002).

C. Therapies aimed at down-regulating fibroblast function

Excess fibrosis or scarring can be disfiguring and in the case of internal organs, life threatening. Not surprisingly then, considerable effort has been devoted to developing rational treatment strategies for fibro-contractive diseases. Given the central role of the fibroblast in this process, much of this attention has focused on down-regulation of fibroblast function, with cell culture studies in particular providing pharamacological insights.

Ostensibly by increasing intracellular cAMP and cGMP concentrations, phosphodiesterase inhibitors reduce proliferation and collagen synthesis by renal (Preaux et al. 1997; Hewitson et al. 2000) and hepatic myofibroblasts (Preaux et al. 1997; Hewitson et al. 2000). Other potential antagonists include the HMG coenzyme A inhibitors, so-called statins (Kelynack et al. 2002), and pirfenidone (Hewitson et al. 2001). Although highly toxic, lysyl oxidase and prolyl hydroxylase inhibitors have at least established the proof of principle of targeting inhibition of collagen assembly (Franklin 1997). As mentioned above, we now recognise that a number of endogenous autocoids may moderate fibroblast function. Several studies highlight the ability of HGF (Matsumoto and Nakamura 2001), BMP-7 (Zeisberg et al. 2004) and relaxin
(Samuel 2005) to down-regulate collagen synthesis. In the case of relaxin, benefits are at least in part due to increased generation of collagenases in renal (Masterson et al. 2004) and lung (Unemori et al. 2000) fibroblasts.

**VI. Concluding Remarks**

In many respects, the pathologic process of fibrosis in internal organs resembles disordered wound healing in the skin. If we define fibrosis by the presence of an increased density of interstitial collagenous matrix (especially collagens type I and III), the histological picture of fibrosis could be produced by increased production and/or reduced breakdown of collagen, or by the loss of normal cellular content leaving behind the interstitial reticulin (Gonzalez-Avila et al. 1988) (Jones 1996; Hewitson et al. 1998). In each case fibroblasts are central to the process- proliferating and migrating to the site of injury, synthesising ECM and increasing the density of matrix through contraction.
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<th>CLONE</th>
<th>IMMUNOGEN</th>
<th>SPECIFICITY</th>
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<td>ER-TR7</td>
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<td>(Van Vliet et al. 1986)</td>
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<td>Thymic fibroblasts</td>
<td>Fibroblasts, Macrophages</td>
<td>(Ronnov-Jessen et al. 1992)</td>
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<td>5B5</td>
<td>Prolyl hydroxylase</td>
<td>Fibroblasts and other collagen producing cells</td>
<td>(Wilkinson et al. 1992)</td>
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<td>Rabbit polyclonal</td>
<td>Ecto-5'-'Nucleotidase</td>
<td>Fibroblasts, Macrophages</td>
<td>(Bachmann et al. 1993)</td>
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<tr>
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<td>α-smooth muscle actin</td>
<td>Smooth muscle cells, Myofibroblasts</td>
<td>(Darby et al. 1990)</td>
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<td>FSP-1</td>
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<td>Murine fibroblasts</td>
<td>(Strutz et al. 1995)</td>
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Table 1  Fibroblast “Specific “ antisera
Table 2 Principal pathologic situations in which myofibroblasts have been identified.

<table>
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<tr>
<th>PATHOLOGY</th>
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<td>Skin wound healing</td>
<td>(Majno et al. 1971); (Darby et al. 1990); (Hebda et al. 93); (Pattison et al. 1994)</td>
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<td>Pulmonary fibrosis</td>
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<td>Hepatic fibrosis</td>
<td>(Bhathal 1972); (Tanaka et al. 1991); (Schmitt-Graff et al. 1993)</td>
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<td>Tumours</td>
<td>(Ronnov-Jessen and Petersen 1993); (Hewitt et al. 1993); (Takahashi et al. 1994)</td>
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<td>Cardiac overload</td>
<td>(Leslie et al. 1991)</td>
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<td>Renal fibrosis</td>
<td>(Alpers et al. 1994); (Hewitson et al. 1995); (Goumenos et al. 1994); (Essawy et al. 1997); (Vangelista et al. 1989); (Pedagogos et al. 1997)</td>
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References


FIGURE LEGENDS

Fig 1. Generalised model of wound healing and scarring versus pathological fibrosis

Fig 2. Myofibroblasts take up an orientation that lines up with mechanical tension across the wound and parallel to the epidermis. The cells also show signs of contraction with rippling of both the cytoplasm and nucleus. Staining is with anti-α-smooth muscle actin antibody.

Fig 3. Myofibroblasts have prominent cortical microfilament bundles with dense bodies. These are not observed in normal connective tissue fibroblasts. (Reproduced with permission from Darby, Skalli and Gabbiani. 1990, Laboratory Investigation, 63:21-29.)

Fig 4. An example of pathological fibrosis in an organ. This renal biopsy is stained with silver Masson’s shows the expansion of the interstitium caused by fibroblast activation and increased extracellular matrix synthesis.

Fig 5. Staining for the myofibroblast marker, α-smooth muscle actin shows that in the normal kidney biopsy (left) only the arteriole adjacent to the glomerulus is positive, while in the biopsy from a fibrotic kidney (right) there are numerous α-smooth muscle actin-positive myofibroblasts present in the interstitium.

Fig 6. The concentration of BrdU positive cells (brown nuclei) at the wound margin shows evidence of cell recruitment from surrounding unwounded dermis.

Fig 7. MMP13 (collagenase) staining suggests that myofibroblasts are migrating and dissecting off the eschar (left) but also invading from surrounding unwounded dermis (right) where they are seen staining strongly at the wound margin.
**Fig 8.** Myofibroblasts are deleted during granulation tissue resolution by apoptosis. Visible here in granulation tissue apoptotic cells have TUNEL-positive nuclei (left) and in electron micrographs have classical apoptotic nuclear morphology (right). (Electron micrograph reproduced with permission from Darby, Skalli and Gabbiani. 1990, Laboratory Investigation, 63:21-29.)