Tissue and molecular events in human conjunctival scarring in ocular cicatricial pemphigoid

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Summary. Detailed histomorphometric analysis of human conjunctival biopsy specimens has convincingly demonstrated that tissue remodeling of the extracellular matrix (ECM) is an essential and dynamic process associated with conjunctival scarring in ocular cicatricial pemphigoid (OCP). The conjunctival scarring often eventually results in impaired vision and/or blindness. The molecular mechanisms of conjunctival scarring are not completely understood. Accumulating evidence indicates that the early phase of conjunctival fibrosis is linked with an immuno-inflammatory process mediated by cytokines released by activated conjunctival cells and/or by infiltrating cells. Fibrogenic cytokines secreted by inflammatory cells and fibroblasts might actively be involved in remodeling of the matrix within the conjunctival stroma, possibly by regulating the altered metabolism of matrix proteins.

Key words: Ocular cicatricial pemphigoid, Conjunctiva, Fibrosis, Collagen, Transforming growth factor-β1, Heat shock protein 47

Introduction

The conjunctiva is a delicate mucous membrane that covers the undersurface of both eyelids and anterior sclera, and merges with corneal epithelium at the corneoscleral limbus. It consists of an epithelium and underlying substantial proprium or stroma. Approximately five layers of nonkeratinizing squamous epithelium line the outer surface of the conjunctiva, while the underlying stroma is composed of connective tissue and vessels with lymphocytes, plasma cells, mast cells and Langerhan’s cells (Allansmith et al., 1976, 1978; Rodrigues et al., 1981; Foster, 1986; Friedlaender, 1993). The conjunctival epithelium and underlying stroma are connected by a delicate but physiologically and metabolically active epithelial basement membrane zone (BMZ). Auto-antibodies directed against target molecules in the BMZ play a role in the separation of basal epithelium from the underlying basement membrane in the conjunctiva of patients with ocular cicatricial pemphigoid (OCP).

Self-tolerance is usually acquired due to clonal deletion or the inactivation of developing lymphocytes that are potentially harmful to the body (Miller et al., 1990; Hiruma et al., 1992; Asano et al., 1996), thus preventing the immune system from reacting destructively against self-components (i.e. autoimmune disease). OCP is an autoimmune disease, in which a yet unknown initial trigger, in a genetically susceptible individual, provokes loss of tolerance to one or more components of the BMZ, with generation of specific B cell clones, which produce auto-antibodies to those BMZ glycoproteins. These auto-antibodies then bind to their specific antigen(s) in the BMZ to initiate complement activation, resulting in an increased infiltration and/or migration of inflammatory cells, including lymphocytes, neutrophils, monocytes/macrophages and mast cells (Foster, 1986). The subepithelial basement membrane separation in OCP appears to be direct cytotoxic effect, while the infiltrating inflammatory cells probably release fibrogenic mediators, including TGF-β1, PDGF, b-FGF, interleukins, etc, to promote healing but with subsequent scarring.

The conjunctival scarring is an important cause of visual disturbances in cicatricial disorders like pemphigoid and trachoma (Wright, 1986; Conway et al., 1997). Conjunctival scarring is also a major cause of failure of glaucoma filtration surgery (Grierson, 1983; Hitchings and Addicks et al., 1983). In addition, infectious conjunctivitis due to adenovirus, Corynebacterium diphtheriae, and streptococcus may cause conjunctival cicatization. Sarcoïdosis, progressive systemic sclerosis and Sjögren’s syndrome may also develop cicatrical changes. Chronic use of tropical ocular drugs, certain skin and multiple mucous membrane disorders like Stevens-Johnson syndrome, toxic epidermal necrosis, epidermolysis bullosa and pemphigus vulgaris may also clinico-pathologically involve conjunctiva, indistinguishable from ocular
Conjunctival scarring in cicatrical pemphigoid

Pemphigoid (Foster, 1986). Although the exact molecular mechanisms of conjunctival fibrosis are not known, it would appear to be similar to the fibrosis in other human diseases (Konomi et al., 1981; Bensadoun et al., 1996; Razzaque et al., 1998b, 1999a). An increased deposition of extracellular matrix (ECM), predominantly collagens, in the substantia propria results in conjunctival subepithelial fibrosis (Dutt et al., 1996; Abu el-Asrar et al., 1998a). Patients with severe conjunctival fibrosis go blind in about 25% of cases. Hence the development of effective anti-fibrotic agents is a major therapeutic challenge with enormous therapeutic potential.

We summarize herein the existing information about conjunctival fibrosis. Based on our current knowledge we also discuss studies, which might provide new insight into the pathophysiology and potential therapeutic interventions for this problem.

**Ocular cicatrical pemphigoid**

Mucous membrane pemphigoid is a family of relatively uncommon vesiculobullous diseases in which oral mucosa is the most consistently affected area of the body; although other mucous membranes (including conjunctiva) may also be involved, the mouth is the most consistently affected. Conjunctival involvement in mucous membrane pemphigoid is typically termed OCP. OCP may occur simultaneously with oral lesions but can present exclusively in the conjunctiva, without the involvement of other mucous membranes. In OCP, BMZ separation leads to subepithelial vacuoles and bulla formation without any acantholytic changes in the overlying epithelium. Metaplastic changes of the lining epithelium and an increased infiltration of chronic inflammatory cells in the substantia propria with stromal fibrosis are common histological changes found in OCP. A genetic predisposition has been suggested in the immuno-pathogenesis of OCP, with an association with HLA-DQ81*0301 phenotypes over represented in OCP patients (Ahmed et al., 1991).

OCP is a systemic autoimmune disorder usually characterized by recurrent episodes of inflammation and progressive subepithelial conjunctival fibrosis, with eventual visual loss secondary to keratopathy (Foster et al., 1982; Bernauer et al., 1993a). Ocular involvement in mucous membrane pemphigoid occurs in about 77% of cases (Mondino and Brown, 1981). It is widely accepted that circulating antibodies against conjunctival basement membrane antibodies binds with their antigens and activate the complement cascade to initiate the ocular inflammatory process in OCP (Mondino et al., 1977; Proia et al., 1985; Chan et al., 1993). This statement is based in part on pathological studies of human conjunctival biopsy sections that demonstrate the presence of immunoglobulins and complement proteins along the conjunctival basement membrane. Although circumstantial evidence is provocative, it fails to establish the specific role of these components in the pathogenesis of the disease; circulating antibody against conjunctival basement membrane has not been detected in all OCP patients, while, immunoglobulins (IgG, IgA) and complement have been found to be present along the conjunctival basement membrane in about 67% OCP patients (Franklin and Fitzmorris, 1983; Leonard et al., 1988; Bernauer et al., 1993b). But there are difficulties in evaluating these percentages in view of the wide range of assays used in different studies and the level of their sensitivities in detecting various immunoglobulins and complement.

Multiple interrelated factors play collectively to determine the ability of an auto-antibody to react within and to trigger conjunctival injury. To date, immune complex formation as a result of circulating antibody against an intrinsic conjunctival antigen, present in the BMZ, has been considered the main mechanism in conjunctival injury in OCP, although no concrete information supports the idea that auto-antibodies derived from the circulation is capable of conjunctival injury; but recently it has been shown that normal human conjunctiva, incubated with OCP patient's sera develops subepithelial separation in an in vitro organ culture system (Chan et al., 1999). These in vitro results emphasize a pathogenic role of circulating antibody in OCP.

However, formation of antibody against an antigen not native to the conjunctiva but present there by virtue of a biochemical and/or electrostatic affinity for BMZ components has not been identified. But, immune complex deposits have been shown in the kidney, induced by exogenous antigens planted by electrostatic interactions between glomerular anionic sites and cationic immune reactants. Such antigens include bovine serum albumin (pI=9.0) and cationized ferritin (Vogt et al., 1988; Bass et al., 1990). Moreover, theoretically, cationic proteins derived from inflammatory cells and platelets, as well as antibody (cationized IgG), could serve to bind anionic proteins in the lamina densa zone to induce subsequent immuno-inflammatory cascades (Vogt et al., 1982; Kaseda et al., 1985); a similar phenomenon has been speculated in some forms of immune-mediated renal diseases.

The antibodies directed against BMZ are not well characterized, but antibodies against basement membrane antigens of 230 kDa (desmoplakin) and 160-180 kDa (hemidesmosome) have been detected in OCP patient's sera (Sarret et al., 1989; Niimi et al., 1992; Mohimen et al., 1993; Ghohestani et al., 1996). Recently, studying the sera from patients with active OCP, B4 integrin has been identified as one of the target autoantigens (Kumari et al., 2001). In addition, circulating autoantibodies directed against epiligrin have also reported in selected patients with OCP (Hsu et al., 2000).

The pathogenesis of OCP is not yet clear, but T-cell mediated immunity is also thought to play a major role in the disease process (Mondino et al., 1981; Soukiasian et al., 1992; Elder and Lightman, 1994). It has been
shown that in active OCP, there is a significantly increased infiltration of CD4+ T-helper cells and CD1+ Langerhan's cells. Moreover, the ratio of T-helper/suppressor cells was higher in the conjunctival sections of OCP patients than in those of control subjects (Sacks et al., 1986; Bernauer et al., 1993b; Elder and Lightman, 1994). An increased infiltration of T-cells, local proliferation of macrophages and dendritic cells in conjunctival stroma has also been reported in active OCP patients (Elder and Lightman, 1994; Bodaghi et al., 1997; Razzaque et al., 2001b). All these inflammatory infiltrates might play a role in subsequent conjunctival fibrosis by triggering increased production of certain fibrogenic cytokines responsible for inducing the fibrotic cascade. For example, it has been shown that T cell clones generated from skin affected by scleroderma were CD4+ and produced high levels of IL-4 but not INF-γ upon in vitro activation; IL-4 has been reported to increase proliferation and chemotaxis of fibroblasts, to increase type I collagen, tenasin and decorin synthesis (Wegrowski et al., 1995; Lee et al., 1996; Makhlu et al., 1996; Chizzolini et al., 1998).

Compared to the other well-studied immune-mediated diseases, where rigorous analysis of experimental animal models provides the foundation of our understanding (Yamamoto and Wilson, 1987; Roberts et al., 1989; Bolton et al., 1993; Koyama et al., 1995), the exploration of immunological mechanisms of conjunctival injury in OCP is greatly hampered due to lack of experimental models. Moreover, the scarcity of human conjunctival tissue has restricted research opportunities. Conjunctival tissue is not excised from the eye during routine surgical procedures like cataract surgery; only biopsies for examination of systemic diseases and autopsies have provided a limited source of conjunctiva for scientific investigation.

**Conjunctival scarring and extracellular matrix**

Conjunctival fibrosis is a complex process, which comprises a complex sequence of events, including stromal inflammatory cell infiltration, proliferation of matrix-producing fibroblasts and an increased accumulation of ECM. The phenotype of proliferating fibroblasts in conjunctival scarring and whether there is any trans-differentiation of resident conjunctival cells during the fibrotic process are not yet known. Recently it has been shown that normal conjunctival fibroblasts do not express the proto-oncogene c-myc, while the conjunctival fibroblasts obtained from OCP patients do express c-myc (Hunt et al., 1991), suggesting possible phenotypic changes in OCP fibroblasts. It is likely that c-myc expressing conjunctival fibroblasts might synthesize increased levels of matrix proteins similar to scleroderma fibroblasts (Trojanowska et al., 1988; Feghali et al., 1993). In scleroderma fibroblasts, an elevated expression of c-myc was associated with increased proliferation of scleroderma cells, which are responsible for an increased production of type I collagen (Kahari et al., 1984; Strehlow and Korn, 1998; Ohtsuka et al., 1999). Moreover, an increased level of type I collagen mRNA with increased activity of α2(I) collagen promoter has also been shown in scleroderma fibroblasts, suggesting the enhancement of type I collagen expression at the transcriptional level (Hirayama et al., 1998).

Excessive accumulation of ECM, especially fibrillar collagens type I and type III, is a hallmark of fibrosis in human and experimental lung, liver, kidney and skin fibrosis, possibly due to transcriptional activation of the corresponding genes (Badid et al., 2000; McCrudden and Iredale, 2000). An increased deposition of collagens, mainly type I and type III collagens, has also been detected in conjunctival fibrosis (Dutt et al., 1996; Abu el-Asrar et al., 1998), but the role of other non-collagenous ECM are mostly unknown. We have also found an increased deposition of type I collagen (Fig. 1A,B) and type III collagen (Fig. 1C,D) in fibrotic stroma in conjunctival biopsy sections obtained from OCP patients. In a separate study, increased deposition of type I, III and IV collagens with *de novo* expression of type V collagen has been found in trachomatous conjunctivitis (Abu el-Asrar et al., 1998b). However, the cells producing increased levels of ECM during conjunctival fibrosis have not yet been defined. Recently, we have found that fibroblasts isolated from conjunctiva of OCP patients could produce increase level of type I collagen, type III collagen and tenascin (Colon et al., 2001; Razzaque et al., 2001a).

**Factors regulating conjunctival fibrosis**

Like most of the other human fibrotic diseases, conjunctival scarring is a slowly progressive process, and usually leads to irreversible conjunctival damage, which is a major determinant of morbidity and visual prognosis.

Fibrosis, in general, is an end result of excessive deposition of various ECM proteins due to altered synthesis and/or degradation of ECM components; the abnormal balance of these processes results in the alteration of the structure and function of the involved tissue and/or organs. Generation of ECM is predominantly achieved through the production of collagens, whereas resorption of the ECM is mediated predominantly by the matrix metalloproteinases (MMPs) (Arthur, 1998; Diamond et al., 1998). In addition, tissue inhibitor of metalloproteinase (TIMP) has an active role in the fibrotic process (Iredale, 1997; Trojanowska et al., 1998). As mentioned earlier, increased deposition of collagens contribute significantly to the conjunctival fibrosis in OCP patients, but possible roles of MMPs and TIMPs in conjunctival fibrosis have not been examined to date. Considering a generalized role of both MMPs and TIMPs in other human fibrotic diseases, it is likely that these molecules might have a role(s) in conjunctival fibrosis in OCP patients, although this remains to be proven. In a recently published study, overexpression of
MMP-1 and MMP-3 has been demonstrated in conjunctivochalasis fibroblasts (Li et al., 2000). ECM degradation is an integral part of wound healing, and a delicate balance between matrix-degrading enzymes (MMPs), and their inhibitors (TIMPs) has evolved to ensure adequate removal of damaged matrix components (Kahari and Saarialho-Kere, 1997) during the healing process; a disparity in the process could lead to an excessive accumulation of matrix proteins. Our preliminary observation shows an increased expression of certain MMPs (mainly MMP-1 and -14) and TIMPs (mainly TIMP-1, -2 and -3) in fibroblasts isolated from conjunctiva of OCP patients, compared to the control conjunctival fibroblasts (unpublished observations).

Various fibrogenic cytokines, including interleukins (IL-1, 4, 6) and TGF-β1, have the potential to mediate both human and experimental fibrosis. Of these, TGF-β1 is a highly studied molecule, expressed at high levels during tissue remodeling, and it greatly affects the formation of connective tissue, possibly by stimulating the transcription of ECM genes (McGowan, 1992; McWhirter et al., 1994; Karsenty and Park, 1995; Vindevoghel et al., 1998; Jimenez and Saitta, 1991; Chen et al., 2000). Both in vitro and in vivo studies have convincingly shown that modulation of TGF-β1 could suppress collagen production and subsequently modulate the fibrotic process (Franklin, 1997; Hori et al., 1998; McCormick et al., 1999). Recently, in acutely inflamed human conjunctiva, a significant increase of TGF-β1 and 3 has been shown in the substantial propria in OCP patients (Elder et al., 1997); using in situ hybridization, it was shown that conjunctival epithelial cells and fibroblasts could produce increased level of TGF-β in conjunctival sections obtained from OCP patients. Moreover, a fibrogenic role for all three isoforms of TGF-β is reported during the development of mouse conjunctival fibrosis (Cordeiro et al., 1999a). It has also been shown that the exogenous application of TGF-β2 could reverse the anti scarring effects of Mitomycin-C in mouse conjunctival fibrosis (Cordeiro et al., 1999b). Our preliminary results demonstrated an increased expression of TGF-β1, TGF-β2 and TGF-β3 and their receptors in fibroblasts isolated from conjunctiva of OCP patients, when compared to normal conjunctival fibroblasts (Razzaque et al., 2001a). Also, a parallel correlation between increased expression of TGF-β1 and elevated production of interstitial collagens was seen in fibroblasts isolated from conjunctiva of OCP patients (Razzaque et al., 2001a). Besides, when fibroblasts from normal conjunctiva were treated with TGF-β1, it could

**Fig. 1.** Immunohistochemistry for type I collagen (A, B) and type III collagen (C, D) in conjunctival sections obtained from control individuals (A, C) and OCP patients (B, D). Note that compared to control conjunctival sections (A, C), an increased submucosal deposition of type I collagen (B) and type III collagen (D) in conjunctival sections of OCP patients. x 20
induce the expression of interstitial collagens. The addition of a TGF-β receptor type II blocking antibody could inhibit the effect of TGF-β1-induced collagen expression by fibroblasts isolated from normal conjunctiva in culture (unpublished observations). Although TGF-β is certainly a key cytokine, the tissue remodeling cannot be explained by a single factor. It involves a complex network of interacting cytokines and other fibrogenic factors.

PDGF is another cytokine that is involved in mediating and modulating the complex biological processes that occur during tissue injury (Ross, 1989; Johnson et al., 1993; Abboud, 1995). PDGF is a family of closely related proteins that are synthesized as approximately 30 kDa disulfide-bonded dimers of A and B chains, PDGF AA, PDGF AB, and PDGF BB (Ross et al., 1986; Hedin, 1992). Moreover, two distinct types of PDGF receptors have been cloned and characterized (Heidaran et al., 1993). PDGF-α receptor recognizes both chains of PDGF and binds all the three isoforms, whereas PDGF-β receptor recognizes predominantly the B chain and binds with high affinity to the BB homodimer. Binding of PDGF dimers to the extracellular part of the receptor induces activation of signal transducing proteins to exert the pathobiologic functions. Extensive human and experimental studies in the renal fibrotic diseases have revealed upregulation of PDGF and its receptors (Gesualdo et al., 1991; Iida et al., 1991; Tang et al., 1996). And an association between the expressions of PDGF components with subsequent fibrosis has been found in the kidney. Moreover, neutralizing the in vivo effects of PDGF by exogenous agents has been shown to modulate subsequent renal fibrosis (Johnson et al., 1992; Ludewig et al., 2000). Hence, PDGF is assumed to be an important molecule involved in the fibrotic process, and the potential exists for its role in conjunctival fibrosis as well. In fact, up-regulation of the expression of PDGF has been reported in the conjunctival sections obtained from OCP patients (Bernauer et al., 1993b). The cells responsible for increased expression of PDGF in OCP are as yet unidentified, but earlier studies in other fibrotic organs have shown that infiltrating macrophages and proliferating fibroblasts/myofibroblasts are the main sources of PDGF (Gesualdo et al., 1994; Homma et al., 1995).

Cytokines and growth factors, including TGF-β1, IL-1, TNF-α and PDGF have been demonstrated in elevated levels in clinical and experimental fibrotic disorders (Gesualdo et al., 1991; Iida et al., 1991; McGowan, 1992, McWhirter et al., 1994, Tang et al., 1996; Cordeiro et al., 1999a). PDGF is a powerful mitogen and chemoattractant for fibroblasts. In addition, the TGFβ and IL-1 responses occur largely through the interaction of the AA homodimer of PDGF via PDGFα receptor (Pinzani et al., 1995, Wang et al., 2000). PDGF-AA/PDGF-β pathway has been shown to mediate the mitogenic response of lung fibroblasts in pulmonary fibrosis and keloid scarring (Haïsa et al., 1994, Osornio-Vargas et al., 1996); and the potential exists for a role of PDGF system in conjunctival fibrosis as well. A detailed study is needed to understand the mechanism of action of PDGF in the conjunctival fibrotic process; studying different isoforms of PDGF, its receptors, and localizing their sources and identifying the signal transducing molecules involved in the cascade should provide a better knowledge about the specific role of PDGF in conjunctival injury.

Heat shock protein 47 (HSP47), is a collagen-specific molecular chaperon involved in the biosynthesis and secretion of procollagens (Nagata, 1998). Substantial in vivo studies indicate that HSP47 is an important fibrogenic factor in various organs, including skin, lung, liver and kidney (Kawada et al., 1996; Razzaque and Taguchi, 1997, 1999a-c; Cheng et al., 1998; Razzaque et al., 1998a-d, 2000). These reports are of interest, because abnormal metabolism of collagens is partly responsible for structural damage of the conjunctiva. In view of the apparent pathophysiological role of HSP47 in other fibrotic diseases, where it is assumed to play a fibrogenic role by increased synthesis and/or assembly of procollagens (Razzaque et al., 1999b), it is likely that HSP47 has a pathological role in conjunctival fibrosis. In fact, a role of HSP47 has been suggested during embryonic corneal development and morphogenesis (Tanaka et al., 1996). We are currently studying the possible role of HSP47 in conjunctival scarring and our preliminary results showed an increase in the conjunctival expression of HSP47 in sections obtained from OCP patients, with its elevated expression in the stromal fibroblasts (Fig. 2). In addition, fibroblasts isolated from conjunctiva of OCP patients showed significantly increased expression of HSP47 at both mRNA and protein level, when compared with normal conjunctival fibroblasts (Fig. 3). A positive correlation between increased expression of HSP47 and excessive accumulation of collagens was seen in conjunctival sections obtained from OCP patients (Razzaque et al., 2001a). Furthermore, when normal conjunctival fibroblasts were treated with recombinant TGF-β1, we found an upregulation, in the expression of HSP47. This biological activity of TGF-β1 could be blocked by TGF-β type II receptor neutralizing antibody; which suggest that TGF-β1 is one of the important molecules that might, at least partly, regulate the bio-activity of HSP47 in the conjunctival fibroblast (unpublished observations). An increased expression of HSP47 by conjunctival fibroblasts might play an important role in increased assembly/synthesis of collagens, and thereby could play a role in submucosal fibrosis in OCP patients.

Local changes of microenvironment might explain why lesions in OCP are not always generalized, rather confined to a specific site. Conjunctival fibroblast-secreted products, including various cytokines and growth factors, might modulate local microenvironment, and thus could eventually facilitate and/or intensify the local immuno-inflammatory responses and subsequently conjunctival injuries. Our preliminary study
demonstrated that conjunctival expression of macrophage colony-stimulating factor (m-CSF) was up-regulated in sections of conjunctiva obtained from OCP patients, and was associated with an increased accumulation and local proliferation of macrophages (Razzaque et al., 2001b). Increased expression of m-CSF might facilitate an increased proliferation of macrophages, and thereby could exaggerate inflammatory process in the conjunctiva of OCP patients. In addition, macrophage derived various fibrogenic factors including TGF-β1 and PDGF could subsequently play roles in scarring process of conjunctiva in the later stages of disease in OCP patients.

**Conclusion**

In OCP, early immuno-inflammatory events are followed by chronic cicatricial changes with subepithelial fibrosis, as a result of excessive deposition of matrix proteins. The destructive process of OCP is caused by fibrosis beneath the conjunctival epithelium. Progression of subepithelial fibrosis results in fornix foreshortening due to shrinkage of conjunctiva, and ultimately leads to formation of symblepharon, meibomian duct obstruction, and eventual lacrimal duct obstruction.

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**Fig. 2.** Immunohistochemistry for HSP47 in conjunctival sections obtained from a control individual (A) and an OCP patient (B). An increased number of submucosal HSP47-expressing cells (arrow) are seen in the section of conjunctiva obtained from an OCP patient (B), x 20

**Fig. 3.** Immuno-expression of HSP47 in fibroblasts isolated from conjunctiva of a control individual (A) and an OCP patient (B), revealing an increased cytoplasmic expression of HSP47 in fibroblasts obtained from conjunctiva of an OCP patient (B), x 20
compression with reduced tear flow (Foster, 1986). The fibrosis also causes deformity of the lid and global architecture. Corneal involvement is usually a result of trichiasis, abnormal blinking and decreased tear film production. If untreated, the cornea can be completely scarred and keratinized, and with blindness the result.

Fibrosis is characterized by the progressive accumulation of ECM proteins, mainly due to transcriptional activation of these proteins. It is assumed that activated conjunctival fibroblasts are the main source of the matrix proteins that constitute the scar tissue. Although many details of conjunctival fibrogenesis in OCP remain to be elucidated, inflammatory events initiated locally by the activation of complement cascades might facilitate the activation and proliferation of fibrogenic cells in the lamina propria. Fibrogenic factors including TGF-β released by these activated cells, by autocrine and/or paracrine functions might turn on the fibrotic cascade, resulting extensive remodeling of the conjunctival tissues. Understanding the precise cellular and molecular events that lead to fibrosis in OCP patients might establish an effective therapeutic strategy to treat otherwise untreatable conjunctival scarring.

**Future directions**

Over the past several years, remarkable progress has been made in understanding the molecular mechanisms of fibrotic diseases in various tissues and organs. However, very little is known about the molecular mechanisms of conjunctival fibrosis. Generating an animal model for active OCP will be useful and broaden our knowledge about the immunopathogenic mechanisms of this disease. Moreover, the origin and source of all the components of ECM in normal and fibrotic conjunctiva are not yet known. The identification of ECM-producing cells will be important for a better understanding of the mechanism of conjunctival scarring found in various diseases. Advances in molecular biology techniques have allowed us to identify most of the factors and even their roles (to some extent) in the routine biopsy sections. Applying all these molecular biological techniques, identifying regulating factors that initiate the fibrotic cascade in conjunctival fibrosis, and establishing an effective therapeutic strategy by targeting those factors to treat conjunctival fibrosis will be our challenge.

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