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Interplay between IL-1α and TGF-β1 in the normal and hypertrophic wound healing

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ABSTRACT

Wound healing is a complex process, strictly regulated at cellular and molecular levels. The slightest disturbance of this process can lead to the formation of hypertrophic scars (Hscars), a pathological condition of a fibrotic nature. Hscars are characterized by their reddish appearance due to the formation/maintenance of an extensive network of blood vessels (neoangiogenesis). However, the most striking feature of Hscars is excessive collagen deposition, sustained by myofibroblasts that remain in the area despite healing of the injured surface. Many molecules are involved in this process, such as TGF-β1 and IL-1α, molecules with pro-fibrotic and inflammatory activity, respectively. Once established, Hscars may only partially heal over time, resulting in the formation of fibrous cords or contractures that can compromise organ function in its entirety, or only partially. To date, there is no effective treatment against the formation of Hscars. The therapeutic tools available are restricted to the use of ointments, laser treatments, or the use of compression garments. In extreme cases of disabling contractures, surgical excision is the recommended approach. Non-invasive treatments (ointments, local compression, and so on) cause an increase in the expression of IL-1α at the lesion site. The objective of this review work is to characterize the individual action of each cytokine and its concomitant actions when acting in the same temporal space, in a wound healing context

DESCRIPTORS

IL-1 alpha, TGF-β1, Wound healing, Fibrosis and Hypertrophic scars.

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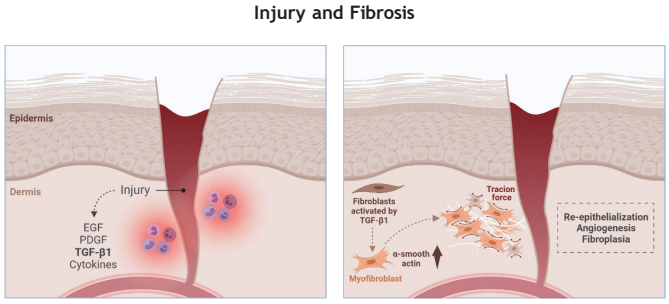
The normal wound healing process

Normal healing is a complex yet dynamic physiological response to injuries and involves four overlapping phases: i) hemostasis (initial phase), ii) inflammatory phase, iii) proliferation phase and iv) remodeling phase[1-4]. Microvascular lesions and leakage of blood fluid into the wound characterize injury (Figure 1). The loss of structural vascular integrity triggers the mechanisms of (initial) hemostasis. This process starts with platelet activation and protein recruitment from the coagulation cascade (intrinsic and extrinsic pathways) and ends with the formation of a temporary matrix, consisting essentially of fibrin-fibronectin[5,6]. The increase in the fibronectin synthesis in this phase is an early event in healing and fibrosis. The fibronectin matrix functions as an initial scaffold for the fixation of collagen molecules also promoting cell adhesion and migration[7].

Figure 1. Physiological response to injury. In response to injury, the inflammatory process is initiated, and several proteins are activated such as fibroblast growth factor (EGF), platelet-derived growth factor (PDGF), TGF-β1, and various cytokines. TGF-β1 has an important role in the activation of fibroblast and myofibroblast promoting collagen production for tissue remodeling. Also, the re-epithelialization, angiogenesis, and fibroplasia contribute to the restoration of the dermis and epidermis.



Platelet activation can lead to the binding, aggregation, and release of fibrinogen, as well as other ECM proteins, for example, trombospondin-1 (TSP-1)5. Thrombospondin-1 (TSP-1) is a 450 kDa matrix glycoprotein that has anti-angiogenic, pro-apoptotic and immunomodulatory properties. It is also a major endogenous activator of the TGF-β (transforming growth factor-β) profibrosing factor[5,8]. At the bruised spot, activated fibroblasts change their phenotype turning into myofibroblasts, a cell type with intermediate characteristics between fibroblasts and smooth muscle cells[9,10].

They also play a central role in wound contraction[11]. However, others mesenchymal cells may also originate myofibroblasts such as circulating fibrocytes, bone marrow mesenchymal stem cells, smooth muscle cells, cells from the epithelial-mesenchyme transition, etc[12-15]. The inflammatory phase begins immediately and in response to tissue trauma[5,6,16]. This phase is characterized by increased capillary permeability and cell migration at the wound site[6]. Local production of pro-inflammatory cytokines (IL-1, for example) and the recruitment of immune cells (macrophages, neutrophils and lymphocytes) are responsible for the mechanisms for the elimination of cell debris and pathogens[5]. Immediately after the injury (or within a few hours), neutrophils migrate to the lesioned site in response to chemotactic agents, such as platelet-derived growth factor (PDGF), TGF-β1, fibroblast growth factor (FGF)[17], as well as IL-1 and various other cytokines and growth factors. Neutrophils are the cells responsible for controlling infection in the wound through the production and release of various potent antimicrobial molecules, such as eicosanoids, cationic peptides as well as proteinases (elastase, cathepsin, proteinase 3 and activator of plasminogen type urokinase)[18].

Macrophages are responsible for the production of inflammatory cytokines such as TNF-α and IL-1[19], which, in turn, activate the nuclear factor-NFkB pathway that stimulates the production of MMPs[20]. In addition to creating/maintaining the inflammatory microenvironment, macrophages are involved in the production of other growth factors such as vascular endothelial growth factor (VEGF), TGF-β1, basic FGF (bFGF), PDGF and keratinocyte growth factor (KGF), responsible for the migration and proliferation of fibroblasts and angiogenesis. The control of the inflammatory response is very important because prolonged inflammation can damage healthy tissue[5]. Macrophages play a crucial role in the transition from the inflammatory to the proliferative phases[21] and their depletion disrupts wound healing, leading to the formation of fibrotic tissue[22]. The proliferative phase begins after the 3rd day and ends between 2 to 4 weeks after the injury[3]. This phase is characterized by re-epithelialization, angiogenesis, and fibroplasias[19]. The high cell density (fibroblasts and macrophages) and the presence of a vast vascular network immersed in a matrix rich in collagen, fibronectin and hyaluronic acid are the most important characteristics of the granulation tissue[23]. In this phase, the fibroblasts actively secrete fibronectin, a multifunctional non-collagenous protein detected both in the plasma soluble form and as a constituent of the insoluble fraction of the ECM. It also plays an important role in the myofibroblast transformation process[24].

Fibronectin is an essential element in this phase because not only does it form an initial scaffold for cell migration, but it also serves as deposition/assembly of matrix proteins. It has several sites of adhesion that allow it to bind to various molecules such as collagen, fibrin and proteoglycans, as well as cells via integrins[25]. Despite being encoded by a single gene, fibronectin is found in different isoforms, which is the result of the alternative splicing of their domains EDA, EDB (for extra-domain A or B) and the domain IIICS (type III connecting segment). The variant form of fibronectin ED-A is a critical cofactor in the process of phenotypic change of fibroblasts into myofibroblasts and expressed parallel to that of ASMA (α-SMA)[12, 26-28].

Over expression of α-SMA - a protein that participates in the formation of stress fibers - is the most important phenotypic feature of myofibroblasts[12]. The restoration of the epidermis begins with the migration and proliferation process of keratinocytes, stimulated by TGF-β1 and followed by neo-epithelialization and restoration of the basement membrane (BM)[3,6].

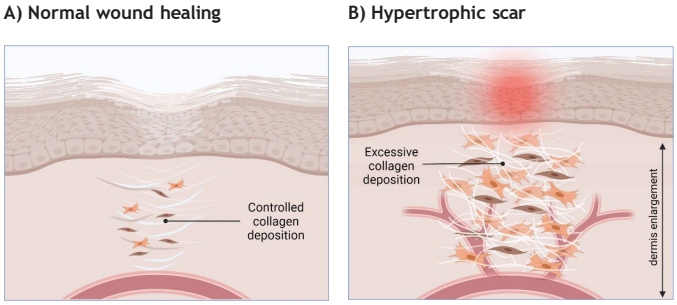
Angiogenesis is stimulated by different cytokines produced by macrophages, and myofibroblasts such as TGF-β1, FGF, and VEGF[3,6,29,30]. Vascularization is a process which can take place from the 4th day to 3 weeks[31]. During their migration, fibroblasts proliferate and deposit matrix proteins, forming the granulation tissue which is essential for normal healing[6,32]. The granulation tissue replaces the temporary fibrin/fibronectin matrix, to form a more stable ECM that serves as a physic-chemical scaffold for cell adhesion and proliferation[31].

Fibroblasts and myofibroblasts - the predominant cells in this phase - are responsible for the production of collagen and other matrix molecules (fibronectin, glycosaminoglycans, hyaluronic acid, etc.)[6,31]. Tissue remodeling is the last phase of the healing process and extends from 6 to 24 months or more, after the initial injury. It is a period of reorganization of the ECM and more particularly immature fibers of the type III collagen and mature fibers of type I collagen[33,34]. At this stage, vascular regression, disappearance of the granulation tissue and formation of new ECM elements are observed, especially type I collagen and fibronectin6. These events are produced by the action of PDGF and TGF-β1[6]. During this phase the turnover of the ECM is intensified through an increase in the expression of MMPs.

Hypertrophic scars (hscars)

Tissue repair is a complex biological process. The slightest disturbance in this process can lead to the formation of hypertrophic scars (Hscars) which occur only in humans[35]. The Hscars are a fibro-proliferative disorder resulting in excessive deposition of collagen and other matrix molecules (Figure 2)[4,36,37]. This is a consequence which can be observed following deep dermal lesions including burns, abrasions, surgeries, infections, acne, folliculitis, trauma, etc[4,37,38]. Unfortunately, the complete etiology of Hscars remains an unknown subject because of the lack of validated preclinical model[39].

Figure 2. Difference between normal wound healing and hypertrophic scar. A) In normal wound healing, there is a controlled production of collagen and matrix molecules. B) On the other hand, in the hypertrophic scar (Hscar) can be observed an increase in the number of fibroblasts and myofibroblast resulting in an excessive deposition of collagen which leads to the enlargement of the dermis. Also, Hscars present higher neovascularization than normal wound healing.

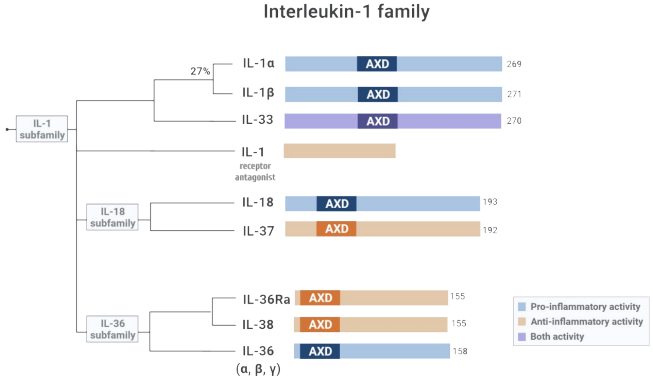


Topographically, Hscars are higher and harder than normal skin (excessive matrix deposition), but do not protrude from the original surface of the wound. Due to extensive neovascularization, Hscars are characterized by their reddish coloring. Generally, Hscars can cause pruritus, pain and joint contractures[37,40]. Histologically, Hscars are characterized by residual but persistent inflammation[41], an increase in the number of fibroblasts / myofibroblasts organized as clusters, the persistence of blood vessels, and abnormal deposition of collagen. At the cellular level, fibroblasts and myofibroblasts play a central role in Hscars[4,42].

Interleukin 1 alpha (IL-1α)

Interleukin 1-alpha (IL-1a) is a pro-inflammatory cytokine that belongs to a family of cytokines, classified as Interleukin 1 (IL-1)[65-68]. Currently, the IL-1 family consists of 11 members: 7 agonists (IL-1α, IL-1ß, IL-18, IL-33, IL-36α, IL-36ß, IL-36γ), 3 antagonist receptors (IL-1Ra, IL-36Ra, IL-36) and 1 anti-inflammatory cytokine (IL-37) (Figure 3)[65-68].

Figure 3. Representation of the interleukin-1 family and its activity. The interleukin-1 family is subdivided into three subfamilies (IL-1, IL-18, and IL-36). Each subfamily member shares the same AXD consensus sequence position.

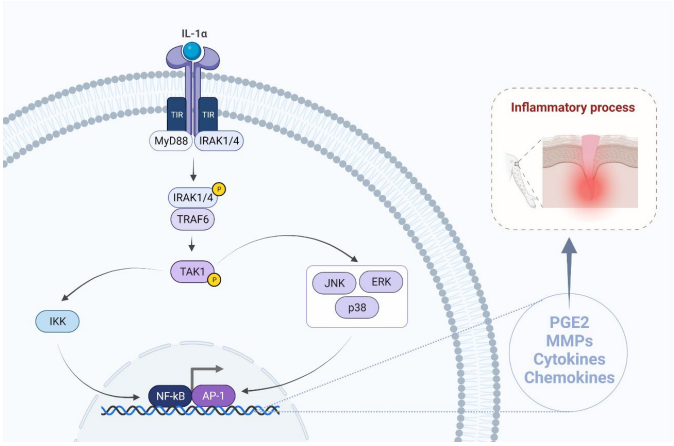


IL-1α and IL-1ß are the most studied members and share only 27% of homology in their amino acid sequence. Nonetheless, their biological activities are similar[69]. Despite the low homology percentage between the primary structure of IL-1α and that of IL-1ß, their molecules have similar three-dimensional structures formed by ß- strand, composed of 12 ß-strands[70]. Differences between IL-1α and IL-1ß are more dependent on their cell source and production mechanism than possible differences after the binding of these cytokines to receptors. IL-1α and IL-1ß are encoded by two different genes located on chromosome 2[71].

They are synthesized as a biologically inactive 37 kDa pro-peptide by an unconventional pathway independent of the Golgi system-endoplasmic reticulum apparatus[71]. IL-1α is produced an as pro-peptide and unlike IL-1ß, the cleavage of the pro-peptide (pro-IL-1α), generates two bioactive fragments: the N-terminal fragment lL- 1α (IL-1α-NTP) and the mature C-terminal IL-1α fragment, both having almost the same affinity to their receptor[72]. The pro-peptide IL-1α is constitutively expressed in cells and can be cleaved by proteases such as calpain. The precursor and the mature form of IL-1α are biologically active[73-76].

Mature IL-1α is rarely secreted or detected in body fluids. However, the IL-1α precursor can be found in cell membranes of various cell types which may explain their cell-cell paracrine signaling[77,78]. Pro-IL-1α contains a nuclear localization signal sequence (NLS) which is conserved in the N-terminal fragment after cleavage by calpain or other proteases[72] and the two fragments can move towards the nucleus. Nonetheless, the exact mechanism of this displacement remains unknown[72]. However, the full-length IL-1α protein can also bind to the receptor and trigger signaling79. IL-1 receptors form a family of 10 proteins with tyrosine kinase activity[66,68,80]. Despite the number of receptors involved in the signaling of IL-1 family proteins, only IL-1R1, IL-1RAcP (IL-1R3) and IL-1Ra receptors are involved in IL-1α signaling (Figure 4)[68,81].

Figure 4. Signaling pathway of IL-1. The binding of the IL-1 to its receptor activates the interleukin-1 receptor kinase (IRAK) cascade which promotes the release of the nuclear factor kB (NF-kB) and the activator protein (AP-1) to the nucleus for transcription of several molecules involved in the inflammatory process. Abbreviations: TIR: Toll-and IL-1r-like domain, MyD88: Factor of differentiation 88 myeloid, TRAF6: Tumor necrosis factor-associated factor 6, TAK1: TGF-ß activated protein kinase, JNK: c-Jun N-terminal kinase, ERK: Extracellular signal-regulated kinases, IKK: Inhibitor of nuclear factor ß kinase, PGE2: Prostaglandin E2, MMPs: Matrix metalloproteinases.



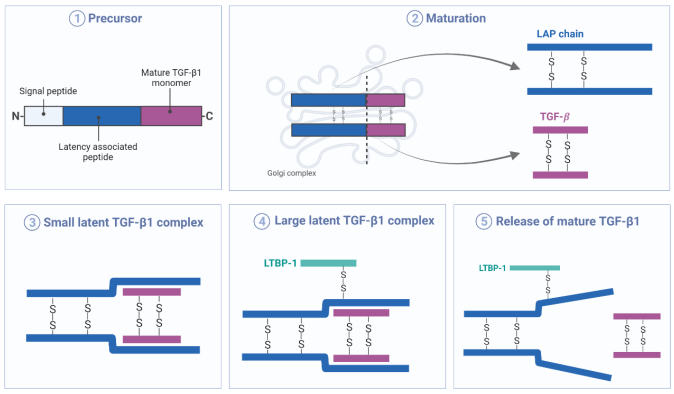
The binding of IL-1α to the IL-1R1 receptor leads to the formation of a complex that forms a heterodimer with the IL-1RAcP receptor (IL-1R3), the accessory protein which serves as co-receptor for signal transduction, downstream of the IL-1α/IL-1R1 complex[79,82]. The IL-1α/IL-1R1/IL-1RAcP trimer (IL-1R3) has highly conserved domains, called Toll- and IL-1R-like (TIR),

responsible for the recruitment of intracellular signaling molecules such as the factor of differentiation 88 myeloid (MYD88) and IL-1 receptor-associated protein kinase 4 (IRAK4)[79,81-89]. IL-1 can be activated by other signaling pathways such as p38, JNK and ERK[79,80,82]. Activation by IL-1 stimulates the synthesis of NO, PGE2, cytokines, chemokines, MMPs and other molecules involved in the inflammatory process[84-86]. Despite being strongly involved in the inflammatory process and also in cancer, interest in studying IL-1alpha has only grown in recent years. Little is known about the regulation of its production, as well as its bioavailability[76].

Transforming growth factor beta 1 (TGF-ß1)

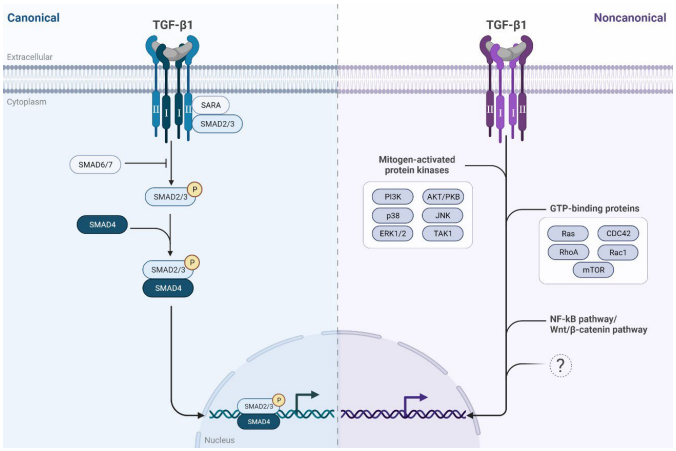
Transforming growth factor beta (TGF-ß) is the prototype of a superfamily protein with structural and functional similarities[43] comprising more than 30 members in mammals[44-46]. About 33 different genes have been linked to TGF-ß family proteins and these proteins[47] are ubiquitously expressed in virtually all human tissues with a very broad spectrum of functions[45]. The TGF-ß family proteins play a key role in several physiological processes from the embryonic phase to adulthood[45]. At the cellular level, TGF-ß family proteins regulate, for instance, proliferation, differentiation, apoptosis, cytoskeletal organization, adhesion, and cell migration. In humans, TGF-ß family proteins include TGF-ß[1-3], activins, inhibins, nodal, growth and differentiation factors (GDFs), and bone morphogenetic proteins in humans (BMP)[2,46,48]. TGF-ß1 was the first member of the family to be identified. Together with the ß2 and ß3 isoforms, they are the most studied in humans. Up to now, TGF-ß1 is the most potent pro-fibrotic cytokine known[14,46]. The activity of TGF-ß1 is strongly regulated at the post-transcriptional level (activation)[49,50]. TGF-ß1, like most TGF-ß family proteins, is synthesized as a broad precursor of about 390-412 amino acids, with an N-terminal domain (signal domain), one pro-domain and the C-terminal domain[43]. The precursors are cleaved inside the Golgi apparatus and the C-terminal fragment (110-140 amino acids) is released[51]. After maturation, TGF-ß1 has a spatial conformation that allows its non-covalent binding to the N-terminal portion of the pro-domain, called latent-associated peptide (LAP). LAP covalently binds to an ECM protein, named latent TGF-ß-binding protein 1 (LTBP-1)[43, 52], in a protein complex called latent large complex (LLC)[50, 52]. In case of tissue injury, the LAP is cleaved and the TGF-ß is released (Figure 5).

Figure 5. TGF-ß1 synthesis. The precursor of TGF-ß1 is constituted by three domains. In the maturation process, the homodimer pro-TGF-ß1 is cleaved inside the Golgi complex and the TGF-ß1 is separated from the latency-associated peptide (LAP) chain. Afterward, the small latent TGF-ß1 complex (SLC) is formed by noncovalent bonds between LAP and TGF-ß1 chains to maintain TGF-ß1 inactive. The SLC binds with the latent TGF-ß1 binding protein (LTBP-1) by disulfide bond generating the large latent TGF-ß1 complex (LLC). At last, LLC is connected to several proteases and integrins changing its initial form and promoting the release of mature TGF-ß1 form.



TGF-ß is activated by two different mechanisms in which integrin plays a very important role[50, 53]. The canonical signaling pathway of TGF-ß activation involves two receptors with serine/threonine kinase activity: TßRI (ALK5) and TßRII, which forms a heteromer capable of activating Smad proteins (Figure 6)[50, 54, 55].

Figure 6. Canonical and noncanonical signaling pathways of TGF-ß1. In the canonical pathway, TGF-ß1 binds to the transmembrane receptor complex (TßRI and TßRII) and it activates Smad2/3 protein by Smad anchor for receptor activation (SARA) signaling. After, the common-mediator Smad (Co-Smad) Smad4 is recruited and forms a complex which is transported to the nucleus and initiates the transcription process. The noncanonical pathway is a Smad-independent pathway in which the TGF-ß1 signal is transmitted to the nucleus for transcription by other pathways such as mitogen-activated protein kinases (MAPK), small GTP-binding proteins, NF-κB pathway, Wnt/ß-catenin pathway, among others. Abbreviations: PI3K: Phosphoinositide 3-kinase, AKT/PKB: Protein kinase B, JNK: c-Jun N-terminal kinase, ERK1/2: Extracellular signal-regulated kinases, TAK1: TGF-ß activated protein kinase, CDC42: Cell Division Cycle 42.



However, TGF-ß can be activated differently, through another type of receptor, called ALK1, whose effects are antagonistic to those of the classical pathway, and which leads to the degradation of the receptor[56, 57]. In addition to TßRI and TßRII receptors, various cell types express co-receptors such as endoglin, betaglycan and CD109 receptor[58-60]. TGF-ß1 can be activated by other non-conventional pathways such as MAPK, Rho, PI3K-AKT, p38 and JNK MAP kinases, TGF-ß activating kinase (TAK1) and focal adhesion kinase[61, 62].

TGF-ß1 participates in all stages of the healing process and is the most potent cytokine as it stimulates the production of type I collagen in fibroblasts[6]. The increase in collagen deposition and the increase in constitutive TGF-ß1 signaling are the two hallmarks of fibrosis[63, 64]. Increasing in the intracellular concentration of Smads proteins in myofibroblasts also highlights the key role of TGF-ß1 in fibrosis.

The combined action of IL-1α and TGF-ß1 in the wound healing

At cellular level, TGF-ß1 plays a major role in development, differentiation, and repair processes[51, 87-89]. In the wound healing context, the pro-fibrotic role of TGF-ß1 is corroborated by thousands of studies that show, among other things, its powerful pro-fibrotic action, *in vivo* and *in vitro*[90, 91]. TGF-ß1 induces the overexpression of CTGF/CCN2[91], a downstream mediator of TGF-ß1, which in turn stimulates the production of type I collagen, a major cause of fibrosis[91-93]. In addition, TGF-ß1 stimulates the differentiation of fibroblasts into myofibroblasts in conjunction with serum response factor (SRF), which is responsible for activating the expression of the ACTA2 gene, the gene coding for α-SMA[94, 95]. While the fibrotic role of TGF-ß1 is widely documented / corroborated in the literature, that of IL-1α in fibrosis remains a controversial topic as the work is very contradictory. In studies on the epithelial-mesenchyme transition, Doerner and Zuraw[96] compare the

fibrotic effects of IL-1 and TGF-ß in human fibroblasts, some studies show that IL-1 acts pro-fibrotic by stimulating collagen synthesis[97, 98]. Wettlaufer, Scott[99] described inhibition of IL-1 (via inhibition of caspase-1, the enzyme responsible for its release from the cell) as being able to cause dedifferentiation of myofibroblasts into fibroblasts by a mechanism that plays on the decrease in expression α-SMA, thus showing a possible pro-fibrotic role of IL1. However, many studies also report an anti-fibrotic role of IL-1α. These studies were performed using liver fibroblasts (stellate cells)[100], pulmonary cells[101] as well as dermal[102] and cardiac fibroblasts[103]. Inoue, Obayashi[100] demonstrated that IL-1α is able to negatively modulate α-SMA gene expression and increase MMP expression, leading to reduced fibrosis. Despite the controversy surrounding the anti- or profibrotic action of IL-1α in patients with Hscars who have undergone traditional therapy (compression garments, laser, etc.), there is an important 'local' expression of IL-1α at the level of the scar. The individual action of IL-1α and TGF-ß1 during the various biological processes is well described in the literature but there is very little work targeting the concomitant actions of these two cytokines. Most of this work focuses on the effects of TGF-ß1 versus IL-1 beta (and not the alpha form). Regarding studies involving IL-1α, the main goal of the work was to find a protein that could link the signaling pathways of IL-1α and TGF-ß1. This protein appears to be the activating protein kinase 1 of TGF-ß (TAK1/ MAP3K7). Initially described as an intermediate of the TGF-ß1 and BMP signaling pathway, TAK1 also activates transcription factors of the NFkB pathway. However, the role of TAK1 in TGF-ß1 signaling is controversial. For example, Sowa, Kaji[104] have demonstrated that TAK1 can activate the p38 protein and modulate a TGF-ß1 response by a Smad-independent pathway. However, the work of Stopa et al.[105] show that IL-1α and IL-1ß are able to inhibit the expression of CTGF/CCN2, a potent stimulator of collagen synthesis that acts downstream of the TGF-ß pathway, through increased of gene expression of the Smad7 protein, a negative regulator of the TGF-ß1 canonical pathway[106, 107]. For this IL-1α phosphorylates Smad3 atypically[108]. The concomitant action of IL-1α and TGF-ß1 during healing was mainly explored during the initial phases of healing (inflammatory phase) and not in the remodeling phase. Mia, Boersema[101] demonstrated, for example, that IL-1ß is able to counter the effects of TGF-ß1 in dermal and pulmonary fibroblasts via the positive modulation of the expression of certain MMPs (MMP-1, -2, 9 and 14) and the stabilization of the activation of the ACTA2 gene (α-SMA). The antagonistic effects between IL-1α and TGF-ß1 and the importance of the balance (ratio) between these two cytokines have been described by Shephard, Martin et al[109]. In human dermal fibroblasts they have demonstrated that IL-1α opposes the effects of TGF-ß1 by decreasing transcription of CTGF/CCN2, via Smad2[102]. Van Nieuwenhoven, Hemmings[103] reported that cardiac fibroblasts co-stimulated with IL-1α and TGF-ß1, showed a decrease in α-SMA expression and TGF-ß1 induced contractile capacity of cells. Understanding the mechanisms involved in the cross-talk between the IL-1α and signaling pathways in the healing context can provide us with valuable information and may open the way for new therapies.

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