Microbes and Infection, 1, 1999, 1275–1282

Bidirectional regulation of macrophage function by TGF-β

Gillian S. Ashcroft

Oral Infection and Immunity Branch, National Institute of Craniofacial and Dental Research, National Institutes of Health, Bethesda, MD 20892, USA

ABSTRACT – The dual role of transforming growth factor-beta (TGF-β) in modulating macrophage function is an important concept gaining increasing recognition. In addition to its role as a 'macrophage-deactivating' agent, TGF-β functions as a monocyte activator, inducing cytokine production and mediating host defence. These functions are context-dependent, modulated by the differentiation state of the cell, the local cytokine environment, and the local levels of TGF-β in itself. In general, during the initial stages of inflammation, TGF-β locally acts as a proinflammatory agent by recruiting and activating resting monocytes. As these cells differentiate specific immunosuppressive actions of TGF-β predominate, leading to resolution of the inflammatory response. Increasing our understanding of the bidirectional regulation of macrophage function will facilitate prediction of the ultimate outcome of modulating TGF-β levels in vivo. © 1999 Éditions scientifiques et médicales Elsevier SAS

TGF-β / macrophage function / deactivation / reactivation / bidirectional regulation

1. Introduction

The classical role of macrophages in phagocytosis and as accessory antigen-presenting cells has gradually been extended to encompass their functions in maintaining tissue homeostasis and mediating inflammatory and immune responses [1]. Transforming growth factor-beta (TGF-β) has emerged as a ubiquitous, global modulator of cellular responses including proliferation, migration, and differentiation [2, 3]. Monocytes/macrophages secrete TGF-β which, in turn, regulates numerous responses such as monocyte activation, cytokine production, host defense, and chemotaxis [4–6]. Beyond the accumulating evidence implicating TGF-β as a potent immunosuppressive and 'macrophage-deactivating' agent [7], recent studies have highlighted the bidirectional effect of this cytokine on monocyte/macrophage function [2, 8, 9]. Specifically, the action of TGF-β on these cells is dependent upon the state of cellular differentiation, the cytokine milieu, and the concentration gradient of TGF-β itself [10, 11]. Largely through recent studies focussing on its role in experimental models of disease and the study of transgenic models, the dual role of TGF-β in modulating monocyte/macrophage function has gained greater understanding.

2. TGF-β: monocyte expression and activation

The generic term 'TGF-β' refers to the three isoforms, TGF-β1, -2, and -3, which have been identified in mammalian species [12]. The isoforms share 64 to 82% amino acid sequence homology and in specific biological assays are largely interchangeable; however, each is encoded by a separate gene and unique promoter, and they show distinct spatial and temporal differences in expression [12, 13]. TGF-β1 is the most abundant isoform in tissues, and is at its most concentrated in platelets [14], whereas TGF-β2 is present in body fluids such as saliva and breast milk, with TGF-β3 being the least abundant of the three. Since TGF-β1 is the predominant isoform present at sites of inflammation/ injury, and is the major isoform secreted by circulating monocytes and tissue macrophages, the majority of studies on the inflammatory response have concentrated on this isoform [4, 15]. However, it is important to

realize that specific isoforms show overlapping and differential effects on hematopoietic cells, albeit in terms of potency [16].

A critical step in the regulation of TGF-β activity at the cellular level is in its secretion from the cell and subsequent activation. Monocytes constitutively express TGF-β mRNA; however, as in other cell systems, mRNA and protein levels of TGF-β do not necessarily correlate [4, 6, 17]. Notably, activated blood monocytes and alveolar macrophages produce increased protein levels of TGF-β secondary to secretion of preformed peptide, as opposed to enhanced transcription [4, 15]. Newly released TGF-β peptide is rendered inactive due to its association with a 75-kDa glycosylated latency-associated peptide (LAP) and the latent TGF-β binding protein (LTBP) [18]. Platelets release the large latent complex (TGF-β covalently associated with LAP and LTBP) in addition to the small latent complex consisting of TGF-β and LAP, which is released during clot dissolution at the sites of injury [19]. The function of LTBP as an anchoring protein localizing TGF-β to matrix may be crucial to the subsequent presentation of TGF-β to activating proteins such as plasmin and thrombospondin, and may be pivotal to the specific spatial localization and concentration gradient of TGF-β [20].

Mechanisms of TGF-β activation include low pH, irradiation, binding to thrombospondin, and proteolysis involving plasmin, and the mannose-6-phosphate (M-6-P) receptor [21, 22]. In addition to the secretion of TGF-β, cells of the monocyte/macrophage lineage are involved in the autocrine and paracrine activation of TGF-β *(figure 1)*. Specifically, the M-6-P receptor, transglutaminase, plasmin, and the urokinase receptor coordinate the activation of TGF-β by stimulated peritoneal macrophages, and are themselves modulated by TGF-β [23, 24]. Moreover, other proinflammatory cytokines secreted by inflammatory cells, such as interferon-gamma (IFN-γ), may modulate monocyte activation of TGF-β, suggesting that monocytes may regulate the local levels of active TGF-β, and that this occurs in a multistep fashion [25]. Subsequently, activated TGF-β may act locally on numerous cell types including autocrine effects on inflammatory cells, and paracrine effects on epithelial and mesodermally derived cells. The production of extracellular matrix by mesenchymal fibroblasts in response to TGF-β results in a fibrotic response in numerous tissues such as lung following bleomycin treatment, and cutaneous wounds following doxorubicin treatment [26, 27]. Alveolar macrophages are the predominant cell type in lung to secrete active TGF-β during bleomycin-induced fibrosis, an activation process involving plasmin [27]. Similarly, macrophages in arthritic synovium constitutively generate TGF-β in its active form [28].

In some circumstances, monocyte production and activation of TGF-β may have detrimental effects, not only by stimulating excessive fibrosis but also by enhancing survival of intracellular pathogens [29, 30], likely by reducing nitric oxide and reactive oxygen intermediates [31]. Specific protozoa and bacteria have developed survival strategies whereby infection of macrophages induces the secretion of active TGF-β, the latter reducing the effectiveness of macrophages to kill the pathogen [30, 32].

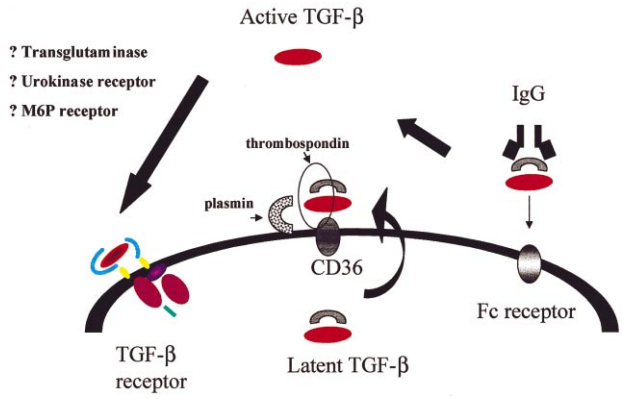

Figure 1. Activation of TGF-β. Macrophages are involved not only in the secretion of TGF-β but also in its activation. Latent TGF-β secreted by these cells binds to the thrombospondin/CD36 receptor complex and is activated by membrane-bound plasmin. In addition, TGF-β bound to IgG released from plasma cells is activated via Fc receptors. Other factors potentially involved in macrophage activation of TGF-β include the urokinase receptor and transglutaminase. Active TGF-β binds to the TGF-β type I and II receptor complex which then directs downstream signalling by Smad proteins and MAP kinases.

A further level of regulation of TGF-β activity lies in the expression of the specific type I (53 kDa) and type II (70–80 kDa) transmembrane receptors. These serine-threonine receptors are responsible for signalling by the TGF-β superfamily members, with the type II receptor binding ligand with high affinity, recruiting the type I receptor to the complex, and leading to phosphorylation of type I receptor on a cluster of glycine and serine residues known as the GS domain [33–35]. TGF-β2 binds with only low affinity to the type II receptor and for high affinity binding requires the type I receptor or the proteoglycan betaglycan [36]. Recently, cytoplasmic transducing molecules known as Smads (42–60 kDa) have been discovered which are involved in TGF-β signal transduction from receptor to the nucleus [35]. Three distinct functional groups of Smads exist at the present time which have been implicated in TGF-β superfamily signalling: pathway-dependent signal-transducing Smads 2 and 3; the common mediator Smad4, and the inhibitory Smads 6 and 7 [35, 37]. While the key Smad proteins involved in macrophage signal transduction are only beginning to be explored, preliminary evidence implicates Smad3 in TGF-β-mediated macrophage activation (Ashcroft et al. in press). Future studies will determine whether the relative ratios of the pathway-restricted Smads and the inhibitory Smads are involved in regulating macrophage responses to TGF-β. Indeed, clear functional distinctions between the two highly homologous proteins Smads 2 and 3 have been reported in human cells of epithelial origin [38].

Current literature suggests that the balance of membrane TGF-β type I and II receptors does indeed play a major role in determining whether inflammatory cell responses to TGF-β are activating or deactivating, and that this balance is determined in part by cell differentiation.

Resting blood monocytes constitutively express approximately 400 high-affinity type I/II TGF-β receptors which render these cells extremely sensitive to stimulation by TGF-β [39, 40]. Activation and differentiation of these cells by TGF-β itself, other cytokines, or bacterial lipopolysaccharide (LPS) leads to a marked reduction in receptor expression and a subsequent decrease in sensitivity to TGF-β1 [10]. Thus, receptor modulation during cellular differentiation provides one mechanism for a bidirectional role of TGF-β, and may help to explain the paradox of TGF-β acting as both a proinflammatory agent and as a factor contributing to the resolution of inflammation.

3. Proinflammatory effects of TGF-β

At the sites of tissue injury, the initial action of platelet degranulation releases a concentrated source of local TGF-β1 [14], and this observation implicated TGF-β as an early mediator of the inflammatory response. In vitro, femtomolar concentrations of TGF-β induce the most potent chemoattractant response observed by human circulating blood monocytes [40, 41]. This in vitro chemoattractant effect has been shown in vivo to effect the recruitment of monocytes to the sites of inflammation/injury, for example intradermal or intraarticular injection of TGF-β stimulates monocyte infiltration and matrix deposition [42, 43]. Moreover, TGF-β mediates monocyte production of cytokines which act as additional mononuclear cell chemoattractants [5, 44]. The influx of monocytes can be further modulated by a number of factors including state of cellular differentiation, receptor expression, the cytokine and proteolytic milieu, and the expression of specific cell surface adhesion molecules [2, 40, 45, 46]. Resting monocytes stimulated by picomolar concentrations of TGF-β respond by upregulating TGF-β transcription and secretion in an autocrine feedback loop; however, this sensitive response is markedly reduced during cellular activation and differentiation [2, 6]. Thus, one mechanism for downregulation of the inflammatory response involves cellular differentiation and reduced responsiveness to TGF-β, resulting in a decrease in cellular chemotaxis.

In order for circulating monocytes to infiltrate the sites of injury/ inflammation, they must first traverse the endothelial basement membrane. The initial mechanism involved is the attachment of circulating inflammatory cells to the endothelial wall, which is mediated by a number of factors, including the expression of specific cell surface integrins *(figure 2)*. TGF-β is a key factor in this response since it enhances the expression of specific integrins such as LFA-1 which binds to endothelial ICAM-1, and $\alpha_3\beta_1$ receptors which mediate monocyte binding to fibronectin, laminin and collagen [46, 47]. In addition to upregulating these integrins which are expressed constitutively on monocytes, TGF-β enhances the expression of the fibronectin receptor $\alpha_5\beta_1$ which enhances attachment to the provisional matrix which is deposited, for example, at the sites of cutaneous wounds [46]. It is important to note that the cytokine milieu at the site of inflammation is

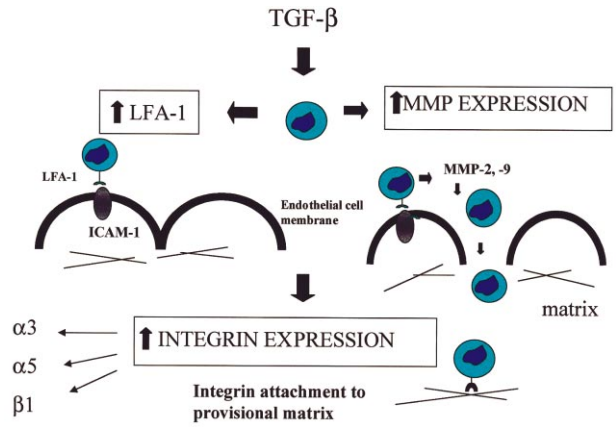

Figure 2. In addition to TGF-β acting as a direct chemoattractant at sites of injury, it facilitates monocyte infiltration to these sites by upregulating LFA-1 on the monocyte cell surface, allowing attachment of the cell to ICAM-1 on the endothelial cell surface. TGF-β mediates the upregulation of monocyte matrix metalloproteinases, degrading the endothelial basement membrane and allowing the monocyte to migrate into the tissue space. Subsequently TGF-β induces integrin expression on the monocyte cell surface which facilitates attachment to the provisional matrix of fibronectin and collagen.

pivotal to these responses, since TGF-β-stimulated expression of monocyte α_5 integrin can be downregulated post-transcriptionally by IFN-γ, leading to inhibition of cell binding to matrix [48]. A further action of TGF-β contributing to monocyte transmigration is its effect on the proteolytic response. Dissolution of the vascular basement membrane, consisting of type IV collagen, fibronectin and laminin, is augmented by the synthesis and secretion by monocytes of matrix metalloproteinases (MMP) 2 and 9 (72-kDa and 92-kDa gelatinases), a process induced by TGF-β [46].

Following adhesion, proteolysis of the endothelial cell membrane, and transmigration in response to TGF-β, chemokines, and other chemotactic stimuli, monocytes are subjected to high concentrations of numerous cytokines at the site of injury/inflammation. Specifically, TGF-β is generated not only by infiltrating monocytes, but also by lymphocytes, fibroblasts, neutrophils, and epithelial cells [4, 15, 49]. In turn, picomolar concentrations of TGF-β stimulate monocytes to transcribe a variety of cytokines such as PDGF, bFGF, IL-6, and IL-1 which modulate cell function [5, 40, 50]. In this context, it is interesting that in the tissues of the TGF-β1 knockout animals, which suffer from rampant multiorgan leukocytic infiltration resulting in a wasting syndrome and death by the fourth week of life [51, 52], infiltrating inflammatory cells may not be activated. This may reflect the absence of a 'cytokine cascade' which is normally influenced by TGF-β [11], particularly in immature monocyte populations. A further activation mechanism involves the induction by TGF-β of Fc gamma receptor III (FcγRIII) or CD16 on the cell surface of monocytes. FcγRIII is constitutively expressed on natural killer cells, on a small subset of

peripheral blood monocytes, and on mature macrophages, but increased in the presence of TGF-β. This process allows circulating and newly recruited monocytes to recognize bound IgG, resulting in enhanced phagocytosis and removal of cellular debris [53, 54]. Intriguingly, TGF-β suppresses monocyte production of GM-CSF, which is an antagonist of TGF-β-induced expression of CD16 [55, 56]. Thus, TGF-β may both directly and indirectly enhance the phagocytic function of monocytes newly recruited to the site of inflammation, which is essential to their function in clearing pathogens and debris.

In vivo evidence for the profound proinflammatory effects of TGF-β emanates from studies where TGF-β activity has been blocked by specific antagonists and the local inflammatory response is markedly reduced. Locally administered antibodies to TGF-β have inhibited tissue destruction, fibrosis, and influx of inflammatory cells in a number of systems, including bacterial cell-wall-induced arthritis and wound healing [28, 57]. Matrix accumulation is also suppressed in experimental glomerulonephritis by the injection of decorin, a proteoglycan which inhibits TGF-β action [58, 59]. Inhibiting the activity of TGF-β1 using topical neutralizing antibodies applied to rodent cutaneous wounds has an anti-scarring effect, associated with reduced monocytic infiltrates [57, 60]. These findings are similar to those observed during fetal wound healing where monocytes are largely absent, endogenous TGF-β levels are low, and healing occurs by scar-free regeneration [57, 61–63]. It is intriguing that the local neutralization of TGF-β1 and TGF-β2 isoforms during rodent cutaneous wound healing reduces the monocyte influx and resulting fibrosis, whereas the application of topical TGF-β3 has a similar effect, suggesting that the modulation of the inflammatory response is possibly isoform-specific [60]. These findings are consistent with the differential appearance of the isoforms during wound healing, with TGF-β3 being expressed at later timepoints [64]. Further evidence for the in vivo proinflammatory action of TGF-β stems from studies using transgenic mice overexpressing the TGF-β1 transgene under the control of the albumin promoter. These animals exhibit elevated circulating levels of TGF-β and progressive hepatic fibrosis and renal disease, characterized by increased matrix deposition and inflammatory infiltrate [65]. Nonetheless, TGF-β's role in inflammation is not limited to the promotion of inflammation, but rather may function as a hinge to reverse these events based on its ability to inhibit macrophage function once they have become activated.

4. Antiinflammatory effects of TGF-β

Based on the many diverse inhibitory effects of TGF-β on the immune response in vitro, numerous in vivo studies have been carried out utilizing the peptide in experimental models of inflammation and immune-mediated disease states. Successful outcomes in these models have confirmed the major role of TGF-β as an in vivo immune/inflammatory suppressor. Clearly, there is a paradox in the action of this cytokine based on its documented proinflammatory activities which is largely resolved by dissociating the effects of TGF-β on resting or nonactivated cells (stimulation) and its converse impact on activated cells (suppression). One major mechanism involved is the downregulation of TGF-β receptor expression as monocytes are activated at the sites of inflammation and differentiate into macrophages. The subsequent decline in sensitivity to TGF-β leads to a distinct temporal sequence of events whereby TGF-β can initially act as a proinflammatory agent and later as a mediator in the resolution of inflammation. Recently, the generation of the TGF-β1 knockout mouse has also shed light on this issue. The multiorgan infiltration of leukocytes in the TGF-β1 knockout mice suggests that, despite the effects on the initiation and amplification of the early inflammatory response, the major role of TGF-β in vivo is in the resolution and downregulation of such a response [66]. How can these effects be explained in light of the known effects of TGF-β on the expression of monocyte cell adhesion molecules? One theory, based on the observation that treatment of knockout animals with TGF-β itself delays inflammation and prolongs survival, suggests that maternal TGF-β deposited in the tissues of the knockout animal serves as a nidus or recruitment factor for initially attracting inflammatory cells, but the subsequent failure to produce TGF-β de novo provides no mechanism to suppress the response, resulting in uncontrolled tissue inflammation [3]. Observations that systemically delivered TGF-β could downregulate endothelial cell adhesion molecule expression and thereby inhibit the inflammatory response, in contrast to the upregulation by local TGF-β of integrins on monocytes, illustrates the importance of TGF-β gradients at the sites of injury/ inflammation [46, 67, 68]. Specifically, adhesion molecule expression is upregulated in the TGF-β knockout mice, in kinetic synchrony with maternal TGF-β in tissues, resulting in the increased cellular adhesiveness to vasculature, which can be blocked by the use of fibronectin peptides that interact with β1 integrins/cell surface proteoglycans [69, 70] or β2 integrins [71]. Moreover, these studies highlight the potential dual function of TGF-β, its effects dependent upon context including local concentrations, route of delivery, and on cell types encountered.

A further mechanism for the antiinflammatory action of TGF-β is via its modulation of cytokines and chemokines elaborated by macrophages. The chemokines MIP-1α and MCP-1 are potent chemoattractants whose production by macrophages is markedly upregulated following stimulation with LPS [72, 73]. TGF-β acts to inhibit the release of MIP-1α, and the expression of MCP-1 possibly via downregulation of c-jun/activator protein-1, thereby contributing to the resolution of the inflammatory response. In this regard, recent studies utilizing TGF-β gene transfer to treat chronic inflammatory disease revealed that the increased TGF-β was associated with a decrease in MCP-1, reduced mononuclear cell infiltration, and amelioration of tissue pathology (Song et al., unpublished). Recently, the role of the macrophage in apoptosis and its contribution to the downregulation of the inflammatory response has been emphasized. During this resolution phase TGF-β produc-

tion by macrophages is stimulated by the ingestion of apoptotic neutrophils, and the increase in TGF-β suppresses proinflammatory cytokine production by macrophages, including IL-8, GM-CSF, and TNF-α [74]. In this context it is interesting that the phenotype of the TGF-β1 knockout animal can be mimicked, in terms of a generalized wasting disorder and tissue inflammation, by raised systemic levels of TNF-α [75]. TGF-β can influence the activity of proinflammatory cytokines by modulating receptor and receptor antagonist expression. TGF-β induces the synthesis by human monocytes of IL-1, which, in turn, mediates the production of IL-1 receptor antagonist [76, 77]. This illustrates the dual role of TGF-β at sites of inflammation: initially it induces the synthesis of the proinflammatory cytokine IL-1, which then triggers the production of its own natural inhibitor. In this context it is interesting that activin A, a member of the TGF-β superfamily, has been shown recently to inhibit the production of IL-1 by monocytes, but enhance the production of IL-1 receptor antagonist [78]. The IL-3 receptor on human monocytes is also downregulated by TGF-β resulting in decreased cellular proliferation [79]. Macrophage 'deactivation' is further achieved by the effects of TGF-β on reactive oxygen and nitrogen intermediates, which are involved in host defense [31]. Hydrogen peroxide production by human monocytes is inhibited and inducible nitric oxide synthase, the enzyme necessary for the production of nitric oxide by activated macrophages, is downregulated by TGF-β both transcriptionally and posttranscriptionally [80]. This effect is demonstrated in the TGF-β1 knock-out mice, where there is increased production of nitric oxide and enhanced expression of nitric oxide synthase [81]. The response of macrophages to TGF-β is dependent upon the initial cytokine that the cell is exposed to and may render the cell unresponsive to other cytokines. For example, preincubation of macrophages with TGF-β abrogates IFN-γ priming for nitric oxide production whereas TGF-β administered 4 h following IFN-γ treatment has no effect [82]. Thus the cytokine milieu to which the macrophage is exposed determines functional activity, and specifically TGF-β renders the cell unresponsive to local activating stimuli. The ability of TGF-β to deactivate or suppress activated inflammatory cells is underscored by in vivo studies of autoimmune and chronic inflammatory disorders. In an animal model of streptococcal cell wall-induced arthritis, systemic delivery of TGF-β [83] or a single dose of 300 mg of plasmid DNA encoding TGF-β injected intramuscularly at the peak of the acute inflammatory phase markedly suppressed the development of chronic joint erosion and inflammation [84]. In addition, systemic delivery of TGF-β inhibits leukocyte influx and decreases the severity of experimental allergic encephalomyelitis [85, 86]. Thus, in several disease models, the administration of systemic TGF-β induces an antiinflammatory response, possibly because of the exposure of endothelium directly to TGF-β in the circulation, resulting in a downregulation of E-selectin [67], as well as neutralization of the TGF-β chemotactic gradient normally radiating from the inflamed tissue. Since the intraarticular injection of anti-TGF-β reduces inflammation and joint destruction in a similar model [28], the concentration gradient of TGF-β at the cellular level and its mode of administration are crucial in determining its ultimate effects. Other studies have confirmed these anti-inflammatory actions of neutralizing TGF-β function locally, for example during wound healing [57, 60] and by inhibiting fibrosis in experimental glomerulonephritis [87].

5. Conclusions

In general terms, it would appear that during the early stages of inflammation, TGF-β locally within a site of injury or inflammation acts as a proinflammatory agent by recruiting and activating resting monocytes. Inhibition of TGF-β function during this stage reduces the degree of inflammation and in some circumstances, reduces downstream matrix deposition and fibrosis. As the recruited monocytes are activated and differentiated, they lose responsiveness to TGF-β, levels of autocrine-stimulated TGF-β decline, and specific immunosuppressive actions of TGF-β predominate such as the induction of IL-1 receptor antagonist, reduction in cytokines, and lymphocyte suppression leading to the resolution of the inflammatory response. Thus, within the inflammatory site and at a cellular level, TGF-β exhibits a temporal pattern of bidirectional activity. Moreover, the effect of exogenous TGF-β can be activating- or inhibitory-dependent upon local versus systemic levels. Targeted mutations of the TGF-β genes have provided evidence that, not only is TGF-β1 critical to life, but that specifically, TGF-β is critical to the regulation of the inflammatory response. In particular, the absence of TGF-β1 leads to multiorgan leukocytic infiltration associated with abnormal regulation of cell adhesion molecules, implying that the key role of TGF-β is in the resolution and suppression of tissue inflammatory cell influx. Paradoxically, circulating levels of TGF-β are increased in the TGF-β1 overexpressing mice and this also leads to renal inflammation and fibrosis. The latter raises a number of questions, since systemic administration of TGF-β leads to an inhibition of inflammation and reduced tissue destruction in specific animal models. The apparent dichotomy between systemic levels of transgene-induced TGF-β and therapeutic delivery appears to be dose-dependent. If TGF-β is delivered at increasing concentrations, beyond what is required for therapeutic efficacy, in autoimmune models renal damage also becomes apparent. In the TGF-β transgenic mice, greater than 6–8-fold increases in circulating TGF-β have been reported, far in excess of therapeutic strategies and injurious to the kidneys [65]. By increasing our understanding of the context-dependent actions of TGF-β, predicting the ultimate outcome of adding or neutralizing TGF-β in specific systems will become less elusive!

References

[1] Bogdan C., Nathan C., Modulation of macrophage function by Transforming growth factor β Interleukin-4 and Interleukin-10, Ann. NY Acad. Sci. (1993) 713–739.

[2] Wahl S.M., Transforming Growth Factor Beta (TGF-β) in Inflammation a cause and a cure, J. Clin. Immunol. 12 (1992) 61–74.
[3] Mc Cartney-Francis N.L., Frazier-Jessen M., Wahl S.M., TGF-β A balancing act, Intern. Rev. Immunol. 16 (1998) 553–580.
[4] Assoian R.K., Fleurdelys B.E., Stevenson H.C., Miller P.J., Madtes D.K., Raines E.W., Ross R., Sporn M.B., Expression and secretion of type beta transforming growth factor by activated human macrophages, Proc. Natl. Acad. Sci. USA 84 (1987) 6020–6024.
[5] Wahl S.M., Mc Cartney-Francis N., Allen J.B., Dougherty E.B., Dougherty S.F., Macrophage production of TGF-β and regulation by TGF-β, Ann. NY Acad. Sci. 593 (1990) 188–196.
[6] Mc Cartney-Francis N., Mizel D., Wong H., Wahl L.M., Wahl S.M., TGF-β regulates production of growth factors and TGF-β by human peripheral blood monocytes, Growth Factors 4 (1990) 27–35.
[7] Tsunawaki S., Sporn M.B., Ding A., Nathan C., Deactivation of macrophages by transforming growth factor-β, Nature 334 (1988) 260–262.
[8] Massague J., The transforming growth factor-β family, Annu. Rev. Cell Biol. 6 (1990) 597–641.
[9] Letterio J.J., Roberts A.B., Regulation of immune responses by TGF-β, Annu. Rev. Immunol. 16 (1998) 137–161.
[10] Mc Cartney-Francis N., Wahl S.M., Transforming growth factor β a matter of life and death, J. Leukoc. Biol. 55 (1994) 401–409.
[11] Wahl S.M., Transforming growth factor beta the good the bad and the ugly, J. Exp. Med. 180 (1994) 1587–1590.
[12] Sporn M.B., Roberts A.B., The transforming growth factor-β past present and future, in: Piez K.A., Sporn M.B. (Eds.), Transforming Growth Factor-β, Chemistry Biology and Therapeutics, New York, New York Academy of Science, 1990, pp. 1.
[13] Roberts A.B., Sporn M.B., Differential expression of the TGF-beta isoforms in embryogenesis suggests specific roles in developing and adult tissues, Mol. Reprod. Dev. 32 (1992) 91–98.
[14] Assoian R.K., Komoriya A., Meyers C.A., Miller D.M., Sporn M.B., Transforming growth factor beta in human platelets, J. Biol. Chem. 258 (1983) 7155–7160.
[15] Grotendorst G.R., Smale G., Pencev D., Production of transforming growth factor beta by human peripheral blood monocytes and neutrophils, J. Cell Physiol. 140 (1989) 396–402.
[16] Jacobsen S.E.W., Ruscetti F.W., Roberts A.B., Keller J.R., TGF-β is a bidirectional modulator of cytokine receptor expression on murine bone marrow cells Differential effects of TGF-β1 and TGF-β3, J. Immunol. 151 (1993) 4534–4544.
[17] Ashcroft G.S., Dodsworth J., Boxte L.E., Tarnuzzer R., Horan M.A., Schultz G.S., Ferguson M.W.J., Estrogen accelerates cutaneous wound healing associated with an increase in TGF-β1 levels, Nat. Med. 3 (1997) 1209–1215.
[18] Wakefield L.M., Smith D.M., Flanders K.C., Sporn M.B., Latent transforming growth factor-β from human platelets, A high molecular weight complex containing precursor sequences, J. Biol. Chem. 263 (1988) 7646–7654.
[19] Grainger D.J., Wakefield L.M., Bethell H.W., Farndale R.W., Metcalf J.C., Release and activation of platelet latent TGF-β in blood clots during dissolution with plasmin, Nat. Med. 1 (1995) 932–937.
[20] Schultz-Cherry S., Murphy-Ulrich J.E., Thrombospondin causes activation of latent transforming growth factor-β secreted by endothelial cells by a novel mechanism, J. Cell Biol. 122 (1993) 923–932.
[21] Harpel J.G., Metz C.N., Kojima S., Rifkin D.B., Control of transforming growth factor-beta activity latency vs, activation, Prog. Growth Factor Res. 4 (1992) 321–335.
[22] Barcellos-Hoff M.H., Derynck R., Tsang M.L.S., Weatherbee J.A., Transforming growth factor-β activation in irradiated murine mammary gland, J. Clin. Invest. 93 (1994) 892–899.
[23] Nunes I., Shapiro R.L., Rifkin D.B., Characterization of latent TGF-β activation by murine peritoneal macrophages, J. Immunol. 155 (1995) 1450–1459.
[24] Falcone D.J., Mc Caffrey T. AMathew J., McAdam K., Borth W., THP-1 macrophage membrane-bound plasmin activity is upregulated by transforming growth factor- beta 1 via increased expression of urokinase and the urokinase receptor, J. Cell Physiol. 164 (1995) 334–343.
[25] Twardzik D.R., Mikovits J.A., Ranchalis J.E., Purchio A.F., Ellingsworth L., Ruscetti F.W., γ-Interferon-induced activation of latent transforming growth factor-β by human monocytes, in: Piez K.A., Sporn M.B. (Eds.), Transforming Growth Factor-β Chemistry Biology and Therapeutics, New York, New York Academy of Science, 1990, pp. 276.
[26] Salomon G.D., Kasid A., Bernstein E., Buresh C., Director E., Norton J.A., Gene expression in normal and doxorubicin-impaired wounds importance of transforming growth factor-beta, Surgery 108 (1990) 318–322.
[27] Khalil N., Corne S., Whitman C., Yacyshyn H., Plasmin regulates the activation of cell-associated latent TGF-β1 secreted by alveolar macrophages after in vivo bleomycin injury, Am. J. Resp. Cell Mol. Biol. 15 (1996) 252–259.
[28] Wahl S.M., Allen J.B., Wong H.L., Dougherty S.F., Ellingworth L.R., Antagonistic and agonistic effects of transforming growth factor-β and IL-1 in rheumatoid synovium, J. Immunol. 145 (1990) 2514–2519.
[29] Connor T.B.J.R., Roberts A.B., Sporn M.B., Danielpour D., Dart L.L., Michels R.G., DeBustros S., Enger C., Kato H., Lansing M., Hiyashi H., Glaser B.M., Correlation of fibrosis and transforming growth factor-beta type 2 levels in the eye, J. Clin. Invest. 83 (1989) 1661–1666.
[30] Champsi J., Young L.S., Bermudez L.E., Production of TNF-alpha IL-6 and TGF-β and expression of receptors for TNF-alpha and IL-6 during murine *Mycobacterium avium* infection, Immunology 84 (1995) 549–554.
[31] Vodovotz Y., Bogdan C., Control of nitric oxide synthase expression by transforming growth factor-beta implications for homeostasis, Prog. Growth Factor Res. 5 (1994) 341–351.
[32] Barral A., Barral-Netto M., Yong E.C., Brownell C.E., Twardzik D.R., Reed S.G., Transforming growth factor beta as a virulence mechanism for *Leishmania braziliensis*, Proc. Natl. Acad. Sci. USA 90 (1993) 3442–3446.
[33] Wrana J.L., Attisano L., Wieser R., Ventura F., Massague J., Mechanism of activation of the TGF-beta receptor, Nature 370 (1994) 341–347.

[34] Wieser R., Wrana J.L., Massague J., GS domain mutations that constitutively activate TβR-1 the downstream signalling component in the TGF-β receptor complex, EMBO J. 14 (1995) 2199–2208.

[35] Baker J.C., Harland R.M., From receptor to nucleus the Smad pathway, Curr. Opin. Genet. Dev. 7 (1997) 467–473.

[36] Rodriguez C., Chen F., Weinberg R.A., Lodish H.F., Cooperative binding of transforming growth factor (TGF)-β2 to the types I and II TGF-β receptors, J. Biol. Chem. 270 (1995) 15919–15922.

[37] Heldin C.H., Miyazano K., Dijke P., TGF-β signalling from cell membrane to nucleus through Smad proteins, Nature 390 (1997) 465–471.

[38] Yanagisawa K., Osada H., Masuda A., Kondo M., Saito T., Yasushi Y., Takagi K., Takahashi T., Takahashi T., Induction of apoptosis by Smad3 and downregulation of Smad3 expression in response to TGF-β in human normal lung epithelial cells, Oncogene 17 (1998) 1743–1747.

[39] Brandes M.E., Wakefield L.M., Wahl S.M., Modulation of monocyte type I TGF-β receptors by inflammatory stimuli, J. Biol. Chem. 266 (1991) 19697–19703.

[40] Wahl S.M., Hunt D.A., Wakefield L., Mc Cartney-Francis N., Wahl L.M., Roberts A.B., Sporn M.B., Transforming growth factor beta (TGF-β) induces monocyte chemotaxis and growth factor production, Proc. Natl. Acad. Sci. USA 84 (1987) 5788–5792.

[41] Wiseman D.M., Polverini P.J., Kamp D.W., Leibovich S.J., Transforming growth factor-beta (TGF-β) is chemotactic for human monocytes and induces their expression of angiogenic activity, Biochem. Biophys. Res. Commun. 157 (1988) 793–800.

[42] Pierce G.F., Mustoe T.A., Lingelbach J., Masakowski V.R., Gramates P., Deuel T.F., Transforming growth factor β reverses the glucocorticoid-induced wound healing deficit in rats Possible regulation in macrophages by platelet-derived growth factor, Cell Biol. 86 (1989) 2229–2233.

[43] Allen J.B., Manthey C.L., Hand A.R., Ohura K., Ellingsworth L., Wahl S.M., Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta, J. Exp. Med. 171 (1990) 231–47.

[44] Luger T.A., Charon J.A., Colot M., Micksche M., Oppenheim J.J., Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor (ETAF) for polymorphonuclear and mononuclear cells, J. Immunol. 131 (1983) 816–821.

[45] Ignotz R.A., Heino J., Massague J., Regulation of cell adhesion receptors by transforming growth factor-beta, Concomitant regulation of integrins that share a common beta 1 subunit, J. Biol. Chem. 264 (1989) 389–392.

[46] Wahl S.M., Allen J.B., Weeks B.S., Wong H.L., Klotman P.E., TGF-β enhances integrin expression and Type IV collagenase secretion in human monocytes, Proc. Natl. Acad. Sci. USA 90 (1993) 4577–4581.

[47] Bauvois B., Rouillard D., Sanceau J., Wietzerbin J., IFN-gamma and transforming growth factor-beta 1 differently regulate fibronectin and laminin receptors of human differentiating monocytic cells, J. Immunol. 148 (1992) 3912–3919.

[48] Bauvois B., VanWeyenbergh J., Rouillard D., Wietzerbin J., TGF-beta 1-stimulated adhesion of human mononuclear phagocytes to fibronectin and laminin is abolished by IFN-gamma dependence on alpha 5 beta 1 and beta 2 integrins, Exp. Cell Res. 222 (1996) 201–217.

[49] Kehrl J.H., Wakefiled L.M., Roberts A.M., Jakowlew S., Alvarez-Mon M., Derynck R., Sporn M.B., Fauci A.S., Production of transforming growth factor-β by human T lymphocytes and its potential role in the regulation of T cell growth, J. Exp. Med. 163 (1986) 1037–1050.

[50] Turner M., Chantry D., Feldmann M., Transforming growth factor β induces the production of interleukin 6 by human peripheral blood mononuclear cells, Cytokine 2 (1990) 211–216.

[51] Kulkarni A.B., Huh C., Becker D., Geiser A., Lyght M., Flanders K.C., Roberts A.B., Sporn M.B., Ward J.M., Karlsson S., Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death, Proc. Natl. Acad. Sci. USA 90 (1993) 770–774.

[52] Kulkarni A.B., Ward J.M., Geiser A.G., Letterio J.J., Hines K.L., Christ M., D'Souza R.N., Huh C., Roberts A.B., Sporn M.B., Mc Cartney-Francis N., Wahl S.M., Glick A.B., Yuspa S.H., Mackall C., Gress R.E., Karlsson S., TGF-β1 knockout mice immune dysregulation and pathology, in: Graham N.G., Shadduck R.K., Levin A.S., Takaku F. (Eds.), Molecular Biology of Haematopoiesis, Intecept, Paris, 1994, pp. 749–757.

[53] Welch G.R., Wong H.L., Wahl S.M., Selective induction of Fc gamma RIII on human monocytes by transforming growth factor-beta, J. Immunol. 144 (1990) 3444–3448.

[54] Phillips J.H., Chang C.W., Lanier L.L., Platelet-induced expression of Fc gamma RIII (CD16) on human monocytes, Eur. J. Immunol. 4 (1991) 895–899.

[55] Hogasen A.K., Hestdal K., Hogasen K., Abrahamsen T.G., Transforming growth factor beta modulates C3 and factor B biosynthesis and complement receptor 3 expression in cultured human monocytes, J. Leukoc. Biol. 57 (1995) 287–296.

[56] Kruger M., Coorevits L., De Wit T.P., Casteels-Van Daele M., Van DeWinkel J.G., Ceuppens J.L., Granulocyte-macrophage colony-stimulating factor antagonizes the transforming growth factor-beta-induced expression of Fc gamma RIII (CD16) on human monocytes, Immunology 87 (1996) 162–167.

[57] Shah M., Foreman D.M., Ferguson M.W.J., Control of scarring in adult wounds by neutralising antibodies to transforming growth factor beta, Lancet 339 (1992) 213–214.

[58] Border W.A., Noble N.A., Yamamoto T., Harper J.R., Yamaguchi Y., Pierschbacher M.D., Ruoslahti E., Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease, Nature 360 (1992) 361–364.

[59] Isaka Y., Brees D.K., Ikegaya K., Kaneda Y., Imai E., Noble N.A., Border W.A., Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney, Nat. Med. 2 (1996) 418–423.

[60] Shah M., Foreman D.M., Ferguson M.W.J., Neutralizing antibody to TGF-β1,2 reduces cutaneous scarring in adult rodents, J. Cell Sci. 107 (1994) 1137–1157.

[61] Whitby D.J., Ferguson M.W.J., Immunohistochemical localization of growth factors in fetal wound healing, Dev. Biol. 147 (1991) 207–215.
[62] Adzick N.S., Lorenz H.P., Cells matrix growth factors and the surgeon, The biology of scarless fetal wound repair, Ann. Surg. 220 (1994) 10–18.
[63] Cowin A.J., Brosnan M.P., Holmes T.M., Ferguson M.W.J., Endogenous inflammatory response to dermal wound healing in the fetal and adult mouse, Dev. Dyn. 212 (1998) 385–393.
[64] Frank S., Madlener M., Werner S., Transforming growth factors β1, β2, and β3 and their receptors are differentially regulated during normal and impaired wound healing, J. Biol. Chem. 271 (1996) 10188–10193.
[65] Kopp J.B., Factor V.M., Mozes M., Nagy P., Sanderson N., Bottinger E.P., Klotman P.E., Thorgeirsson S.S., Transgenic mice with increased plasma levels of TGF-β1 develop progressive renal disease, Lab. Invest. 74 (1996) 991–1003.
[66] Letterio J.J., Roberts A.B., Transforming growth factor-β1-deficient mice identification of isoform-specific activities in vivo, J. Leuk. Biol. 59 (1996) 769–774.
[67] Gamble J.R., Khew-Goodall Y., Vadas M.A., Transforming growth factor-β inhibits E-selectin expression on human endothelial cells, J. Immunol. 150 (1993) 4494–4503.
[68] Lefer A.M., Ma X.L., Weyrich A.S., Scalia R., Mechanism of the cardioprotective effect of transforming growth factor β1 in feline myocardial ischemia and reperfusion, Proc. Natl. Acad. Sci. USA 90 (1993) 1018–1022.
[69] Hines K.L., Kulkarni A.B., Mc Carthy J.B., Tian H., Ward J.M., Christ M., McCartney-Francis N., Furcht L.T., Karlsson S., Wahl S.M., Synthetic fibronectin peptides interrupt inflammatory cell infiltration in transforming growth factor β1 knockout mice, Proc. Natl. Acad. Sci. USA 91 (1994) 5187–5191.
[70] Mc Cartney-Francis N., Mizel D., Redman R.S., Frazier-Jessen M., Panek R.B., Kulkarni A.B., Ward J.M., McCarthy J.B., Wahl S.M., Autoimmune Sjogren's-like lesions in salivary glands of TGF-β1-deficient mice are inhibited by adhesion-blocking peptides, J. Immunol. 157 (1996) 1306–1312.
[71] Diebold R.J., Eis M.J., Yin M., Ormsby I., Boivin G.P., Darrow B.J., Saffitz J.E., Doetschman T., Early-onset multifocal inflammation in the transforming growth factor beta 1-null mouse is lymphocyte mediated, Proc. Natl. Acad. Sci..USA 92 (1995) 12215–12219.
[72] Kitamura M., Identification of an inhibitor targeting macrophage production of monocyte chemoattractant protein-1 as TGF-β1, J. Immunol. 159 (1997) 1404–1411.
[73] Sherry B., Espinoza M., Manogue K.R., Cerami A., Induction of the chemokine β peptides MIP-1α and MIP-1β by lipopolysaccharide is differentially regulated by immunomodulatory cytokines γ-IFN, IL-10, IL-4 and TGF-β, Mol. Med. 4 (1998) 648–657.
[74] Fadok V.A., Bratton D.L., Konowai A., Freed P.W., Westcott J.Y., Henson P.M., Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE2, and PAF, J. Clin. Invest. 101 (1998) 890–898.
[75] Tracey K.J., Wei H., Manogue K.R., Fong Y., Hesse D.G., Nguyen H.T., Kuo G.C., Beutler B., Cotran R.S., Cerami A., Lowry S.F., Cachectin/tumor necrosis factor induces cachexia anemia and inflammation, J. Exp. Med. 167 (1988) 1211–1227.
[76] Turner M., Chantry D., Katsikis P., Berger A., Brennan F.M., Feldmann M., Induction of the interleukin 1 receptor antagonist protein by transforming growth factor-beta, Eur. J. Immunol. 21 (1991) 1635–1639.
[77] Wahl S.M., Costa G.L., Corcoran M., Wahl L.M., Berger A.E.,) Transforming growth factor-β mediates IL-1-dependent induction of IL-1 receptor antagonist, J. Immunol. 150 (1993)b 3553–3560.
[78] Ohguchi M., Yamato K., Ishihara Y., Koide M., Ueda N., Okahashi N., Noguchi T., Kizaki M., Ikeda Y., Sugino H., Nishihara T., Activin A regulates the production of mature interleukin-1β and interleukin-1 receptor antagonist in human monocytic cells, J. Interferon Cytokine Res. 18 (1998) 491–498.
[79] Leveque C., Grafte S., Paysant J., Soutif A., Lenormand B., Vasse M., Soria C., Vannier J.P., Regulation of interleukin 3 receptor a chain (IL-3Ra) on human monocytes by interleukin (IL)-4 IL-10 IL-13 and transforming growth factor β (TGF-β, Cytokine 10 (1998) 487–494.
[80] Warwick-Davies J., Lowrie D.B., Cole P.J., Selective deactivation of human monocyte functions by TGF-β, J. Immunol. 155 (1995) 3186–3193.
[81] Vodovotz Y., Geiser A.G., Chesler L., Letterio J.J., Campbell A., Lucia M.S., Sporn M.B., Roberts A.B., Spontaneously increased production of nitric oxide and aberrant expression of the inducible nitric oxide synthase in vivo in the transforming growth factor β1 null mouse, J. Exp. Med. 183 (1996) 2337–2342.
[82] Erwig L.P., Kluth D.C., Walsh G.M., Rees A.J., Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines, J. Immunol. 161 (1998) 1983–1988.
[83] Brandes M.E., Allen J.B., Ogawa Y., Wahl S.M., Transforming growth factor β1 suppresses acute and chronic arthritis in experimental animals, J. Clin. Invest. 87 (1991) 1108–1113.
[84] Song X.Y., Jin W.W., Klinman D.M., Wahl S.M., Plasmid DNA encoding transforming growth factor-beta 1 suppresses chronic disease in a streptococcal cell wall-induced arthritis model, J. Clin. Invest. 101 (1998) 2615–2621.
[85] Kuruvilla A.P., Shah R., Hochwald G.M., Liggitt H.D., Palladino M.A., Thorbecke G.J., Protective effects of transforming growth factor β1 on experimental autoimmune disease in mice, Proc. Natl. Acad. Sci. USA 88 (1991) 2918–2921.
[86] Racke M.K., Dhib-Jalbu S., Cannella B., Albert P.S., Raine C.S., McFarlin D.E., Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β, J. Immunol. 146 (1991) 1792–1796.
[87] Border W.A., Okuda S., Languino L.R., Sporn M.B., Ruoslahti E., Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1, Nature 360 (1990) 361–364.