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MINI REVIEW

Pathobiology of transforming growth factor β in cancer, fibrosis and immunologic disease, and therapeutic considerations

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Transforming growth factor β (TGF-β) is a highly pleiotropic cytokine that plays an important role in wound healing, angiogenesis, immunoregulation and cancer. The cells of the immune system produce the TGF-β1 isoform, which exerts powerful anti-inflammatory functions, and is a master regulator of the immune response. However, this is context dependent, because TGF-β can contribute to the differentiation of both regulatory (suppressive) T cells (Tr cells) and inflammatory Th17 cells. While TGF-β might be underproduced in some autoimmune diseases, it is overproduced in many pathological conditions. This includes pulmonary fibrosis, glomerulosclerosis, renal interstitial fibrosis, cirrhosis, Crohn's disease, cardiomyopathy, scleroderma and chronic graft-*vs*-host disease. In neoplastic disease, TGF-β suppresses the progression of early lesions, but later this effect is lost and cancer cells produce TGF-β, which then promotes metastasis. This cytokine also contributes to the formation of the tumor stroma, angiogenesis and immunosuppression. In view of this, several approaches are being studied to inhibit TGF-β activity, including neutralizing antibodies, soluble receptors, receptor kinase antagonist drugs, antisense reagents and a number of less specific drugs such as angiotensin II antagonists and tranilast. It might be assumed that TGF-β blockade would result in severe inflammatory disease, but this has not been the case, presumably because the neutralization is only partial. In contrast, the systemic administration of TGF-β for therapeutic purposes is limited by toxicity and safety concerns, but local administration appears feasible, especially to promote wound healing. Immunotherapy or vaccination stimulating TGF-β production and/or Tr differentiation might be applied to the treatment of autoimmune diseases. The benefits of new therapies targeting TGF-β are under intense investigation.

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Transforming growth factor β (TGF-β) is a highly pleiotropic cytokine, that in mammals exists in three isoforms (TGF-β1, TGF-β2 and TGF-β3).[1–3] The importance of TGF-β stems from the fact that it contributes importantly to apoptosis control, angiogenesis, wound healing, immune regulation and tumor biology. The TGF-βs are part of a large superfamily of proteins,[1] but in this review the author will focus only on the three TGF-β isoforms. Practically all cells have receptors for the TGF-βs, and at least one of the isoforms is produced in all tissues.[1–3] The cells of the immune system produce primarily TGF-β1. TGF-β is also normally found in the plasma (TGF-β1 isoform),[1] and bound to extracellular matrix proteins throughout the body.[4] Notably, platelets and bones contain large amounts of TGF-β1.[1–5] Unlike other cytokines, it is secreted in a latent form that can be activated by various mechanisms to exert its effects. Latency is probably essential, in view of the ubiquitous expression of receptors.

The immunological functions of TGF-β have attracted a considerable amount of attention (Figure 1). Indeed, it exerts broad anti-inflammatory and immunosuppressive effects,[3,6] and complete knockout (KO) of TGF-β1 in mice results in autoimmunity and early death from a multi-organ inflammatory syndrome.[7,8] Recent studies[3,6,9] have shown that it is an important differentiation factor (along with IL-2) for some regulatory T cells (denoted Tr or Treg cells) that exert powerful and diverse immunosuppressive effects. However, not all the effects of TGF-β are suppressive because, for

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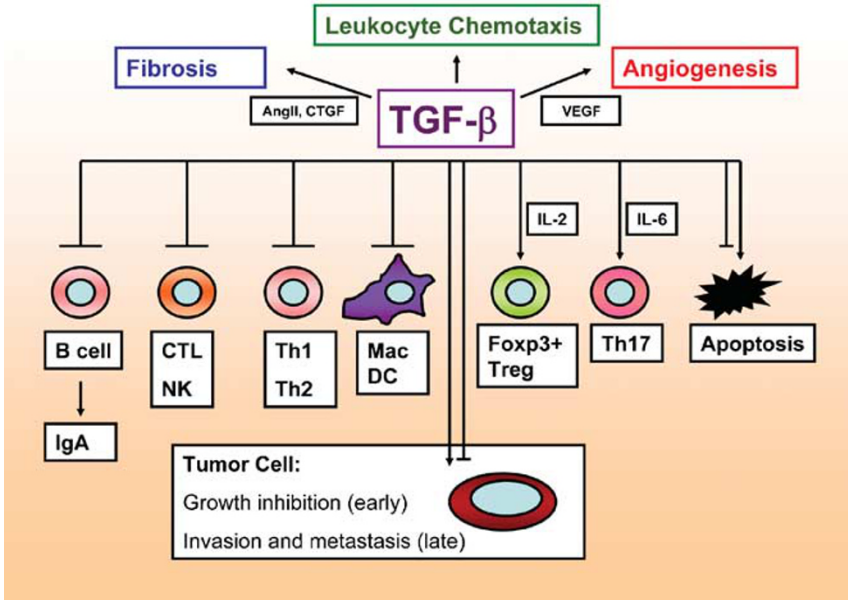

Figure 1 Pleiotropic effects of TGF-β. This cytokine (primarily the TGF-β1 isoform) exerts multiple effects on inflammation, angiogenesis, fibrosis and tumor progression. It stimulates production of VEGF and CTGF, which contributes to angiogenesis and fibrosis, respectively. AngII stimulates production of TGF-β and CTGF, and also promotes fibrosis. It also directly activates the Smad signaling pathway. In the early phase of tissue injury, TGF-β exerts a strong chemotactic effect on leukocytes, but later it exerts primarily immunosuppressive effects on all arms of the immune system (Th cells, CTLs, NK cells, Mac and DCs). TGF-β exerts a general inhibitory effect on B-cell proliferation, differentiation and antibody production, with the exception of IgA. Under optimal stimulatory conditions (where the inhibitory effects of TGF-β are weak or absent), it promotes IgA class switching and IgA production, and contributes to mucosal immunity. In the context of T-cell activation, it promotes Foxp3 expression and regulatory T-cell differentiation (this is enhanced by IL-2). However, if the environment is rich in IL-6, differentiation to an inflammatory Th17 phenotype occurs instead. It can either promote or inhibit apoptosis, also in a context-dependent manner. TGF-β suppresses tumor growth in the early phase of neoplasia, but promotes tumor progression (especially metastasis) at later stages. AngII, angiotensin II; CTGF, connective tissue growth factor; CTL, cytotoxic T lymphocyte; DC, dendritic cell, Mac, macrophage; NK, natural killer cell; Th, T-helper cell; Treg, regulatory T cells; VEGF, vascular endothelial growth factor.

example, in combination with IL-6 it induces the differentiation of Th17 cells,[10] that have been linked to inflammation and autoimmunity. This capacity of TGF-β to induce either immunosuppressive or inflammatory events is context dependent, and must be considered when analyzing its role in disease.[9]

Furthermore, TGF-β plays a major role in cancer (as outlined below), by suppressing tumor growth in the early phase of neoplasia, while promoting tumor progression and metastasis in later phases. Thus, many malignant tumors produce large amounts of TGF-β, but are resistant to its growth inhibitory effects. At the same time, TGF-β produced by tumors depresses anti-tumor immune responses at the level of T-helper (Th) cells, cytotoxic T lymphocytes (CTLs), dendritic cells (DCs), macrophages, natural killer (NK) cell and B cells, while increasing the numbers of Tr cells. These combined immunosuppressive effects diminish the effectiveness of cancer vaccines, and represent a major obstacle to immunotherapy.

In addition to cancer, the production of TGF-β is altered in many pathologic conditions. This can be related to overproduction as in pulmonary fibrosis, cirrhosis, glomerulosclerosis, cardiomyopathy, Crohn's disease, scleroderma and chronic graft-*vs*-host disease (GVHD), or underproduction as in some autoimmune diseases. From this list, it is apparent that targeting TGF-β for therapy is of major clinical interest, and the author will review the many applications and approaches that have been investigated.

TGF-β ACTIVATION AND SIGNALING

The biology of TGF-β is exceedingly complex, and only salient points will be mentioned. It is produced in a latent form consisting of TGF-β and the non-covalently bound latency-associated peptide (LAP; derived from the N-terminal of the TGF-β precursor), that must be released for activation.[1,2,4] *In vitro*, activation is easily accomplished by acidification, but *in vivo* the mechanisms are less clear and several possible modes of activation exist.[4] It is thought that proteolysis by plasmin (a key component of the fibrinolytic system) and other proteases is an important mechanism. At least in some cases, plasmin-mediated activation occurs on the cell membrane. For instance, the mannose-6-phosphate/insulin-like growth factor II receptor (M6P/IGFII-R) can bind LAP-TGF-β on the surface of monocytes and can complex with the urokinase receptor and plasminogen to generate plasmin and activate latent TGF-β.[11] However, other molecules such as thrombospondin 1 (TSP-1), found in platelets and the extracellular matrix, and $\alpha_v\beta_6$ integrin, an

epithelial-cell membrane protein, can also bind LAP-TGF-β and activate it,[12,13] and there is evidence that both are important. TSP-1 is one of the few molecules that can bind both latent and active TGF-β, and it activates this cytokine by inducing a conformational change.[14] Interestingly, the matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) have been implicated as activators of TGF-β.[15] Notably, the CD44 hyaluronan receptor provides a cell-surface receptor for proteolytically active MMP-9, which proteolytically cleaves and activates latent TGF-β. These interactions of CD44, MMPs and TGF-β on the cell membrane appear to affect cancer cell motility, invasion and metastasis.[16]

It should be noted that LAP-TGF-β is usually secreted as a large latent complex consisting of LAP-TGF-β covalently bound to a latent TGF-β-binding protein (LTBP).[4,17] At least three LTBP isoforms bind TGF-β, and it has been proposed that the LTBPs serve as structural components of the ECM and modulators of TGF-β availability. Indeed, LTBPs target latent TGF-β to the ECM. For instance, they concentrate TGF-β to elastin fibrils and fibronectin-rich pericellular fibers.[17] Deficiency of LTBP-4 in mice results in defective elastin structure, developmental abnormalities, emphysema and colorectal cancer.[4,17]

All three TGF-βs use the same receptor and it has three components: type I (RI, or ALK5); type II (RII) and type III (RIII, or betaglycan).[1–3,6] RIII binds TGF-β (all isoforms) and recruits TGF-β to RII, which then phosphorylates RI to form a heterotetrameric serine/threonine kinase complex. In turn, RI phosphorylates Smad2 and Smad3 (receptor-associated Smads (R-Smads)), and the latter form a heteromeric complex with Smad4, which translocates to the nucleus, binds to DNA and regulates transcription (Figure 2). Stimulation of cells with TGF-β can result in the activation or repression of hundreds of genes.[6] In contrast to these Smads, Smad7 inhibits TGF-β signaling. TGF-β also signals through MAPK pathways, and this can lead to a switch from tumor suppression to promotion. Indeed, several signaling molecules are activated by TGF-β (eg, ERK, c-Jun NH_2-terminal kinase (JNK), p38, PI3K, Akt and Rho-like GTPases),[2,3] and there is complex cross-talk between the Smad pathway and other pathways.

In endothelial cells, ALK1 is an additional type 1 receptor, and endoglin acts as a type III-like receptor. The classic TGF-β/ALK-5 (Smad2/3) pathway inhibits endothelial cell proliferation and migration, whereas the alternative TGF-β/ALK1 (Smad1/5) pathway has the opposite effect.[18,19] The role of endoglin is not fully elucidated, but it promotes ALK-1 signaling, and endothelial cells lacking endoglin do not proliferate because TGF-β/ALK1 signaling is decreased and TGF-β/ALK5 signaling is increased.[19] Interestingly, many mutations of either the ALK1 or endoglin genes have been associated with hereditary hemorrhagic telangiectasia.[18]

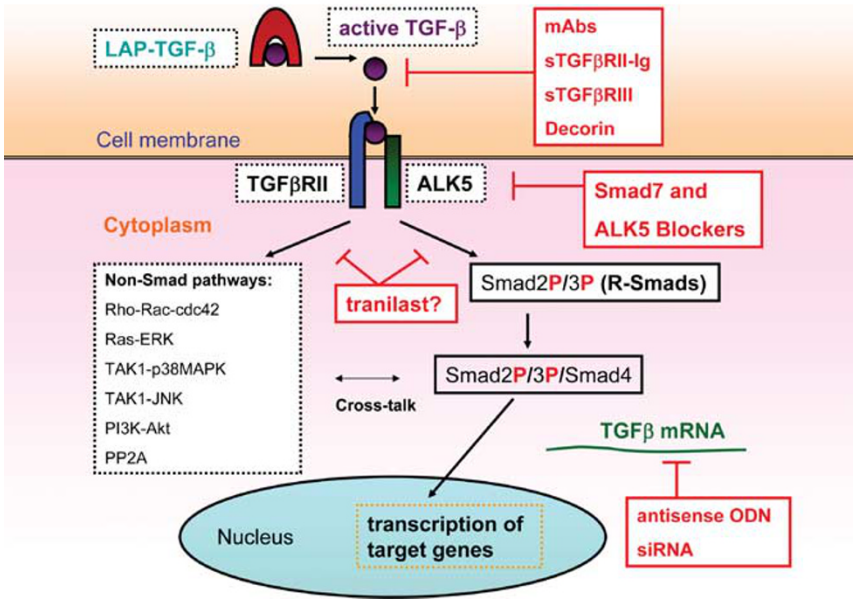

Figure 2 TGF-β signaling and inhibition. TGF-β must be activated by the release of LAP or a conformational change to bind to its signaling receptors. Signals are transduced through a Smad pathway and a number of non-Smad pathways, with complex cross-talk. ALK5 phosphorylates Smad2 and Smad3, which form a complex with Smad4. This complex translocates into the nucleus and (in association with other molecules) binds to DNA, and either activates or represses gene expression. In contrast, Smad7 inhibits ALK5 signaling. Therapeutically, TGF-β activity can be reduced or blocked in many ways (denoted by red boxes), including neutralization of TGF-β with mAbs or soluble receptors, blockage of ALK5 activity with small drugs or Smad7, prevention/degradation of TGF-β mRNA translation with antisense ODNs or siRNA. The mode of action of tranilast is not elucidated, but it inhibits TGF-β production and receptor expression, and reduces signaling through both Smad and non-Smad pathways. ALK5, activin receptor-like kinase 5 (TGF-β type I receptor); LAP, latency-associated peptide; mAbs, monoclonal antibodies; ODNs, oligodeoxynucleotides; siRNA, small inhibitory RNA; RII, type II TGF-β receptor; TAK1, TGF-β-activated kinase 1.

There are several non-signaling receptors of TGF-β, and some were mentioned above (M6P/IGFII-R, TSP-1, $\alpha_v\beta_6$). Recently, we have identified neuropilin-1 (Nrp1) as a novel membrane protein that binds TGF-β (Y Glinka, GJ Prud'homme, manuscript in preparation). Nrp1 is a multi-functional protein known as a receptor for both semaphorins and VEGF,[20] but it is also expressed by cells of the immune system, particularly DCs and Tr cells.[21,22] Nrp-1 and/or its homolog Nrp2 are expressed by most cancers, and appear to contribute to their malignant phenotype.[20] Remarkably, Nrp1 binds both latent and active TGF-β, and we hypothesize that it plays an important role in immunoregulation, through its ability to capture this cytokine.

In the plasma, α2-macroglobulin binds TGF-β and sequesters it in an inactive form.[23] This may be essential to rapidly neutralize circulating active TGF-β. Of note, TGF-β binds to several extracellular matrix components such as TSP-1, decorin, fibronectin, elastin, some collagens and other molecules.[4,17] Thus, the matrix acts as a reservoir for TGF-β (and many other cytokines and growth factors), possibly allowing its release during pathological conditions.

TGF-β AND IMMUNE REGULATION

TGF-β is clearly a master regulator of the immune response, and it exerts inhibitory effects on cells of all arms of the immune system, including Th1 cells, Th2 cells, CTLs, macrophages, NK cells, B cells and polymorphonuclear leukocytes (granulocytes). These multiple effects of TGF-β have been reviewed in the recent literature,[3,6,9] and are highly relevant to autoimmune diseases.[24] Importantly, it also prevents DC maturation.[3,25] On the other hand, TGF-β has potent chemoattractive properties (even at femtomolar concentrations), which can lead to the rapid accumulation of macrophages, granulocytes and other cells at the site of inflammation.[26,27] The inflammatory component is amplified by the ability of TGF-β to induce differentiation of Th17 cells. Indeed, when naïve T cells are activated in the combined presence of TGF-β and IL-6, they differentiate into Th17 cells.[10,28] However, this is highly context dependent, because Th17 differentiation is blocked by a number of cytokines such as IL-2, IL-4, IL-27 and IFN-γ.[10,28–31] The Th17 cells secrete large amounts of IL-17, which sustains acute inflammation by recruiting granulocytes, and also by promoting the secretion of other inflammatory cytokines. Of note, it was recently reported that, in the presence of IL-6, TGF-β1 produced by Tr cells can contribute to Th17 differentiation and, remarkably, some $CD4^+CD25^+Foxp3^+$ Tr cells themselves differentiated into Th17 cells.[32] It appears that TGF-β can exert inflammatory effects early after tissue injury, but is subsequently anti-inflammatory, highlighting its bipolar nature.[9] TGF-β can also either increase or decrease apoptosis of lymphocytes, depending on their phenotype and stage of differentiation.[3] In general, it promotes T-cell survival, but induces apoptosis of immature and resting B cells.

The immunosuppressive effects are most apparent on T cells.[3,6] For instance, TGF-β inhibits both T-cell proliferation, by targeting cell cycle regulators, and IL-2 production by blocking its transcription. The blockade of IL-2 production is highly dependent on Smad3. Other Th1 cytokines, such as IFN-γ, are also inhibited. Furthermore, TGF-β inhibits the differentiation of Th1 cells, Th2 cells and CTLs. In CTLs and NK cells, TGF-β is a strong antagonist of both IFN-γ production and cytolytic activity. TGF-β is also an important negative regulator of B-cell proliferation and differentiation.[33] Interestingly, it inhibits production of most immunoglobulin isotypes, except IgA, which is enhanced.[33,34] This promotion of IgA production corresponds to a protective role of TGF-β in mucosal immunity.

Studies of TGF-β1 KO mice have clearly established the important anti-inflammatory functions of this cytokine.[7,8] These mice die within 3–4 weeks of birth of a multi-organ inflammatory syndrome involving the heart, skeletal muscle, lungs, liver, stomach, pancreas, brain, eyes, salivary glands and other tissues. The inflammatory infiltrate tends to be perivascular and consists of lymphocytes, macrophages and granulocytes, in varying proportion from organ to organ. There is evidence for increased leukocyte–endothelial cell interactions.[8] These mice have evidence of autoimmunity, including circulating anti-dsDNA, anti-ssDNA, other antinuclear antibodies and glomerular immune complex disease. This syndrome shares features with human SLE, Sjögren's syndrome, GVHD and polymyositis. Note that these findings apply only to TGF-β1 KO mice, while TGF-β2 or TGF-β3 KO mice die before or shortly after birth from developmental abnormalities.[4,7] These mice have cleft palate and defective lung development and, in the case of TGF-β2, also defects of the cardiovascular system, bones, urogenital structures and other tissues.

In the absence of TGF-β1, there is lymphoproliferation and markedly increased numbers of activated lymphocytes in lymphoid organs, as well as an increased expression MHC class I and II molecules (which are normally downregulated by TGF-β1).[35] There is also overproduction of several inflammatory cytokines,[35] including MIP-1α, IL-1β, TNF-α and IFN-γ. Anti-CD4 antibodies are protective and, similarly, autoimmune disease is diminished in TGF-$\beta1^{-/-}$ and MHC $II^{-/-}$ (double knockout) mice,[36,37] demonstrating a role for Th cells. As might be expected, mice with severe combined immunodeficiency (SCID mice) are protected from this inflammatory syndrome.

In early studies, TGF-β1 KO affected all cells and it was difficult to confirm that inflammation was dependent on a T-cell defect. However, this question has been addressed with transgenic mice expressing mutant receptors, or more limited genetic deletions. For instance, mice with a deletion of TGF-βRII restricted to T cells develop a severe autoimmune disease and die before 5 weeks of age, and are similar to complete TGF-$\beta1^{-/-}$ mice.[38] Mice with T cells expressing a dominant-negative TGF-βRII receptor have milder disease,[3]

probably because of residual receptor function. Interestingly, mice with T cells unable to produce TGF-β1 (rather than a receptor defect) also have a milder disease, most likely because of production this cytokine by other cells.[39]

In recent years, much attention has been directed at the effects TGF-β on Tr-cell differentiation and function. A detailed discussion of this topic is beyond the scope of this paper, but several recent reviews are available.[3,6,9,40,41] The differentiation of natural Tr cells (nTr cells) of $CD4^+CD25^+Foxp3^+$ phenotype in the thymus appears to be TGF-β independent. In contrast, the differentiation of induced (adaptive) Tr cells (iTr cells) in the periphery is highly TGF-β dependent. Indeed, TGF-β induces the differentiation of $Foxp3^+$ Tr cells from either antigen- or CD3mAb-stimulated $CD4^+CD25^-Foxp3^-$ precursors, and this effect is greatly enhanced by IL-2.[42] Furthermore, in the periphery, TGF-β appears essential for the maintenance of Foxp3 expression, regulatory function and homeostasis of both nTr and iTr cells.[41,43–45] The process of naïve T-cell differentiation to the $Foxp3^+$ iTr phenotype requires both T-cell receptor (TCR) signaling and TGF-β stimulation, and the persistence of this phenotype is dependent on TGF-β.[45] Indeed, in the case of iTr cells removal of TGF-β results in Foxp3 loss *in vitro*; and after adoptive transfer of these cells it is also downregulated rapidly *in vivo* (2 days), except for a small residual population.[45] This loss of Foxp3 expression is much less in nTr cells. There is also evidence that the cytokine profiles of iTr and nTr cells are rather similar, showing low IL-2, IL-4, IL-5, IFN-γ and TNF-α production, but high IL-10 production.[45]

A separate and more controversial issue is whether TGF-β contributes to the suppressive activity of Tr cells. In the case of nTr cells, suppression appears to be dependent on direct cell contact, and has been observed (at least *in vitro*) in the complete absence of TGF-β.[41] The issue of contact dependence, however, does not exclude a role for TGF-β, because LAP-TGF-β has been reported on the membrane of both nTr and iTr cells, and might suppress through a contact-dependent mechanism.[46] Effector T cells engineered to be unresponsive to TGF-β (dominant-negative RII) are resistant to the suppressive activity of Tr cells.[47] Furthermore, the results of some *in vivo* studies, including a recent one with TGF-β-null T cells,[39] reveal an important role for TGF-β as an effector molecule of $Foxp3^+$ Tr cells. Indeed, TGF-β was required to inhibit both Th1 differentiation and inflammatory bowel disease.

It seems likely that Tr cells can suppress by more than one mechanism, depending on the type of immune response or inflammation that is occurring. It is important to add that Th3 cells, which contribute to some forms of immune tolerance (especially when orally induced), produce TGF-β which appears to be their main mode of suppressive action.[48,49] In addition, Tr1 regulatory T cells,[50] which are particularly relevant to the control of inflammatory bowel disease, secrete IL-10 and TGF-β, which both exert important regulatory effects.

TGF-β IN HEALING AND FIBROSIS

Wound repair is a complex multi-phase process, involving inflammatory cell chemotaxis, fibroblast proliferation, collagen and matrix deposition, angiogenesis, reduced matrix degradation by metalloproteinases, remodeling and, in the skin, re-epithelialization.[51–53] Early on, fibroblasts and endothelial cells migrate to the wound site, where they form highly vascular granulation tissue. Fibroblasts in granulation tissue transform into myofibroblasts, and eventually the lesion evolves into a scar with dense collagen, and much reduced vascularity and cellularity. In hypertrophic scars and other pathological fibrosis, there is retention of a high number of fibroblasts and myofibroblasts, and an abundant immature collagen matrix.

TGF-β stimulates most of the processes of wound healing (in collaboration with many other growth factors), and is a major profibrotic factor.[4,53–56] Connective tissue growth factor (CTGF), which is induced by TGF-β, is a major contributor to this process.[53–55] TGF-β also induces endothelin-1, and TGF-β/endothelin-1 interactions may play a role in the development of fibrosis in scleroderma[55] and myocardial disease.[54] When TGF-β is overproduced there is excessive collagen and matrix deposition, culminating in organ dysfunction or failure. Inflammation, ischemia, radiation and toxins are all initiation factors for fibrogenesis, and it can adversely affect the lungs, heart, liver, kidneys and other organs and tissues. This has been demonstrated, for example, by TGF-β gene transfer into the lung,[57] and in bleomycin-induced pulmonary fibrosis.[56] Indeed, cancer chemotherapy with bleomycin results in TGF-β production in the lung, and subsequent pulmonary fibrosis, which is a major adverse effect. The Smads are involved, and Smad3-null mice resist TGF-β-induced pulmonary fibrosis.[57] The importance of fibrosis in human disease cannot be understated, and it responds poorly (if at all) to current therapies.

TGF-β IN CANCER: TUMOR SUPPRESSION *VS* PROMOTION

As mentioned previously, TGF-β can act as either a tumor suppressor or a tumor promoter.[58–64] Suppression of tumor cell growth by TGF-β depends on its ability to upregulate cyclin kinase inhibitors. However, as pre-malignant lesions progress they become refractory to growth inhibition, and begin to produce large amounts of TGF-β. Many malignant tumors have mutated or downregulated TGF-βRII receptor, or other abnormalities of the TGF-β signaling pathways.[65] TGF-βRIII (betaglycan) loss or downregulation has also been linked to breast cancer progression.[66]

With tumor progression, TGF-β becomes a tumor promoter and induces epithelial-to-mesenchymal transition (EMT) by Smad-dependent and -independent pathways.[67–69] EMT is associated with increased secretion of MMPs, which promote tumor intravasation or extravasation. The effects of TGF-β on EMT, tumor growth or metastasis can be dissociated, and might be dependent on different signaling

pathways.[68,69] Interestingly, a mutated TGF-β type I receptor unable to bind Smad2/3, but with a functional kinase domain (capable of activating MAPK and other pathways) showed that deficient Smad2/3 signaling increased the malignancy of a well-differentiated xenografted tumor cell line (higher proliferative index and more malignant histologic features), but suppressed formation of lung metastases by a more aggressive variant of this cell line.[60] This suggests a dominant role for Smad2/3 signaling pathway in both the tumor suppressor and prometastatic activities of TGF-β. These authors also reported that non-Smad signaling pathways, including p38 and JNK, cooperated with TGF-β/Smads in enhancing the migration of metastatic cells, but the non-Smad pathways were not sufficient for inducing metastasis. At any rate, tumor cells respond aberrantly to TGF-β, but TGF-β signaling appears to be required for both invasiveness and metastasis in late-stage tumorigenesis.[70] Furthermore, TGF-β plays an important role as a mediator of interactions between stromal cells and tumor cells, and it regulates the tumor microenvironment.[65]

A recent study in a transgenic murine model of breast cancer revealed that radiotherapy or chemotherapy with doxorubicin increased systemic levels of TGF-β1, and circulating tumor cells and lung metastases.[71] These negative effects of cancer therapy were reversed by the administration of a neutralizing anti-TGF-β mAb (2G7). It has been known since the early 1990s that anti-TGF-β antibodies are protective against experimental breast carcinoma. The intraperitoneal (i.p.) injection of 2G7mAb (like 1D11 it neutralizes the TGF-β1, 2, 3 isoforms) in nude mice reduced the i.p. tumor growth and lung metastases from i.p. injected MDA-231 human breast cancer cells.[72] 2G7 Therapy was associated with increased NK cell activity, and these cells were required for the antitumor effect. The impact of overexpressed TGF-β or activated Smad pathways in the development of a malignant tumor phenotype or metastatic disease has been demonstrated in several transgenic models.[73] In contrast, TGF-β blockade can be protective.[73] However, in an experimental models of mouse mammary carcinoma, conditional KO of TGF-βRII in mammary epithelial cells reduced tumor latency and increased lung metastases,[59] presumably because of the loss of TGF-β's tumor suppressor effect at the earliest stages of disease. These studies suggest that inhibiting TGF-β might not be beneficial in patients genetically predisposed to cancer (since it would remove a tumor suppressor effect), but could ameliorate disease in patients who present with existing TGF-β-producing cancers. TGF-β is produced by a wide variety of tumors (of breast, lung, GI tract, pancreas, ovary, CNS, skin/melanoma and other).[61–65,74] Interestingly, elevated TGF-β1 plasma levels are frequently observed in cancer patients, and this generally correlates with a poor prognosis,[75–79] although these levels are notoriously difficult to measure due to partial platelet degranulation (platelets contain large amounts of TGF-β1).

Importantly, TGF-β stimulates production of CTGF, endothelin-1 and VEGF,[53–55,80,81] and all these factors collaborate in promoting the formation of a vascular and fibrous tumor stroma. Moreover, TGF-β attracts macrophages and other inflammatory cells to the stroma, and these cells secrete various mediators and growth factors that sustain tumor progression.[65] Some of these infiltrating leukocytes differentiate into myeloid suppressor cells, which have broad immunosuppressive properties.[82] TGF-β (produced by either tumor or stromal cells) also directly exerts a wide spectrum of immunosuppressive effects, and induces the differentiation of Tr cells, markedly limiting antitumor immunity. TGF-β inhibits DC functions and interferes with immunization with DC-based vaccines.[83] In accord with the suppressive role of TGF-β, effector $CD8^+$ T cells expressing a dominant-negative TGF-βRII are more effective at eliminating tumors.[84]

One of the most clinically relevant aspects of TGF-β in cancer is its ability to promote bone metastasis.[85–91] This is particularly relevant to breast carcinoma, where bone metastases give rise to debilitating disease. This feature can be targeted for cancer therapy. For instance, in a human breast cancer xenograft model (in nude mice), an ALK5 inhibitor reduced the incidence of both lung and bone metastases.[90] In breast carcinoma, TGF-β acts (at least in part) by stimulating the production of parathyroid hormone-related protein (PTHrP),[86,87,91] which stimulates bone resorption by osteoclasts and the formation of osteolytic bone metastases. The stimulation of PTHrP is dependent on both the Smad and the p38 MAPK pathways of TGF-β signaling.[91] TGF-β is stored in the bone matrix and can be released during osteolysis. Other mediators such as IL-8, CTGF, chemokine receptor CXCR4, IL-11, MMPs and osteopontin have been implicated in the formation of bone metastases.[88] Since TGF-β can upregulate production of CTGF, IL-11 and MMPs, these factors are likely to interact in the metastatic process. CD44 also appears to be important. It binds to matrix hyaluronan and has various tumor promoting activities and, as noted previously, might contribute to the activation of TGF-β.[16]

THE INHIBITION OF TGF-β ACTIVITY FOR THERAPEUTIC PURPOSES

Several large- and small-molecule drugs inhibiting TGF-β are being tested in preclinical and clinical trial (ranging from phase I to III) (Table 1). There have been several reviews of these drugs in the treatment of various conditions,[73,74,92–103] and the author will discuss more extensively some agents that have received less attention, such as tranilast and angiotensin II (AngII) blockers. The therapeutic agents being most studied include mAbs, soluble TGF-β receptors, antisense oligonucleotides (ODNs) and inhibitors of ALK5 (TGF-βRI) (Tables 1 and 2; Figure 2). These TGF-β inhibitory drugs are currently being developed primarily to treat either fibrotic disease or cancer, and some salient findings are summarized in Table 1, although this list is not complete and reviews

Table 1 Examples of drug-mediated specific inhibition of TGF-β

Type/drug target	Diseases targeted	Stage/observations	References
mAbs (pan-TGF-β; TGF-β2 or TGF-β2,3)	Fibrosis Renal disease Cancer Heart disease Radiation injury	Preclinical and clinical phases I–III. Preclinical studies (eg, with mouse1D11 or 2G7 mAb) show positive effects in rodent fibrosis, renal disease, cancer and other, but reveal some adverse effects at high doses (see text)	71–73,92,94,96,100,104
Soluble TGF-βRII receptor constructs (with or without Ig fusion) Target TGF-β1, 3. Protein or gene therapy	Cancer	Preclinical. Effective in cancer models (pancreas, colon, lymphoma, other). Few adverse effects in transgenic mice, and reduced mammary tumor metastases	96,98,100,105
Soluble TGF-βRIII, or P144 peptide. Targets TGF-β (β2 > β3 > β1)	Cancer Fibrosis	Preclinical. Effective against human breast cancer xenografts (MDA-MB-231) in nude mice	66,106–108
Natural TGF-β-binding proteins (decorin, other)	Glomerulopathy Cancer	Preclinical. These proteins do not neutralize all TGF-β isoforms equally, and are not completely specific	94,97,98,102
Nucleic acid-based therapies (antisense ODNs, ribozymes, siRNA, *Smad7*)	Cancer	Antisense ODNs are at an advanced stage of clinical development for high-grade gliomas. Other agents are at a preclinical stage. Delivery of these agents is challenging	92,96,97,100,101
ALK5 inhibitors (GW6604, Ly580276, Ly2157299, SB-505124, SB- 431542, SD-208, several others)	Fibrosis Cancer	Preclinical or phase I. Advantage of orally active small drugs. Have incomplete specificity for ALK5. Predominantly tested in fibrosis models. Can block breast cancer metastasis	73,90,92,94–97,109

ALK5, activin receptor-like kinase 5 (alternative name for the TGF-β type I receptor); mAbs, monoclonal antibodies; ODN, oligodeoxynucleotides; P144, peptide derived from the TGF-βRIII sequence (inhibits TGF-β); RII, type II TGF-β receptor; RIII, type III TGF-β receptor; siRNA, small inhibitory RNA; TGF-β, transforming growth factor β.

containing additional information are quoted. At least a dozen ALK5 inhibitors are being developed by pharmaceutical companies. They have the advantage of being small drugs that can be administered orally, and have shown effectiveness in preclinical models against pulmonary fibrosis, other fibrotic diseases and various malignant tumors. It is interesting that an ALK5 inhibitor was able to ameliorate both early acute fibrogenesis and established fibrosis in a TGF-β-induced model of pulmonary fibrosis.[109] Despite some highly encouraging results, none of the drugs listed in Table 1 is yet approved for regular clinical use.

However, some drugs commonly used to treat other diseases can inhibit TGF-β production and/or action (Table 2). This includes antihypertensive drugs that block the renin–angiotensin–aldosterone (RAS) system. This relates to the fact that AngII stimulates TGF-β production in the kidney and elsewhere.[53,100,147,148] Another important inhibitor of TGF-β is the antiallergic/antifibrotic drug tranilast, used clinically in Japan for many years. These less specific agents may have some therapeutic advantages, at least in some diseases, because they target other aspects of the pathological process and are generally of low toxicity. Furthermore, in the case of cancer, there is a growing realization that drugs that act on a single molecular target may be less effective than those that have multiple targets, because tumor cells mutate rapidly.

ADVERSE EFFECTS OF TGF-β INHIBITION

In view of the many biological effects of TGF-β, including its anti-inflammatory activity, it might be assumed that its inhibition would be highly detrimental. However, contrary to expectations, severe toxicity has not been observed when TGF-β was inhibited by a variety of agents in adult rodents or humans. This paucity of adverse effects undoubtedly results from incomplete inhibition of TGF-β activity, because null mutations in mice, as noted previously, produce a rapidly fatal inflammatory syndrome. Ruzek *et al*[104] observed only minimal pathologic alterations in

Table 2 Examples of multi-action drugs that inhibit TGF-β

Drug	Disease relevance	Stage/observations	References
Tranilast	Allergy Fibrosis Autoimmunity Renal disease Cardiomyopathy Diabetic complications Crohn's disease Cancer	In clinical use for allergy and fibrotic disease in Japan. Inhibits TGF-β, VEGF, PGE2 and several cytokines. Exerts antiproliferative, anticancer and immunosuppressive effects. Low toxicity	103,110–146
Inhibitors of renin–angiotensin system (ACE inhibitors; AT1 receptor blockers)	Hypertension Renal disease Congestive heart failure Pulmonary fibrosis Cirrhosis Scleroderma Muscular dystrophy Cancer	Commonly prescribed antihypertensive drugs, with few adverse effects. Block AngII induction of TGF-β and decrease TGF-β levels	53,139,147–153
HMG CoA reductase inhibitors (statins)	Hypercholesterolemia Cardiomyopathy Fibrosis Renal disease Diabetes	Commonly prescribed cholesterol-lowering drugs, with few adverse effects. Statins inhibit the effects of TGF-β on CTGF induction and fibroblast collagen synthesis	99,154–157
Pirfenidone	Fibrosis Neurofibromatosis Cancer Asthma Multiple sclerosis	This antifibrotic drug has been shown to inhibit TGF-β production. It has been reported to exert anticancer and anti-inflammatory effects. It may be of benefit in pulmonary fibrosis, asthma and multiple sclerosis	100,158,159

ACE, angiotensin-converting enzyme; AngII, angiotensin II; AT1, angiotensin II type 1 receptor; CTGF, connective tissue growth factor; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; PGE2, prostaglandin E2; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

major organs or tissues, or in lymphocyte numbers and function, in mice chronically treated with an anti-TGF-β mAb (1D11) that neutralizes all three isoforms. Doses of up to 50 mg/kg three times a week (resulting in blood levels of 1–2 mg/ml) for 12 weeks were investigated. Similarly, transgenic mice expressing a soluble TGF-βRII-Fc fusion protein that neutralizes TGF-β1 and TGF-β3 did not develop severe pathology and usually had only mild inflammatory lesions, despite lifetime exposure to that protein.[105] There was also no increase in spontaneous tumorigenesis. Nevertheless, some pathological consequences have been noted in other studies, especially when TGF-β inhibitory agents are applied at high doses. Thus, although 1D11 antibody administration was well tolerated at low therapeutic doses, at high doses it induced epithelial hyperplasia of the tongue in mice, associated with dysphagia and weight loss, and increased progression to carcinoma in a model of familial adenomatous polyposis (data presented by Scott Lonning, Genzyme Corporation, at *TGF-β in Cancer and Other Diseases* conference, La Jolla, CA, 2006). In this respect, it is of some concern that TGF-β blockade accelerates the progression of tumors in some genetically determined cancer models.[59] Evidently, the incidence of tumors in patients treated with TGF-β inhibitors should be closely monitored. In addition, inhibition of ALK5 signaling has been reported to induce physeal dysplasia in rats.[160] Undoubtedly, other adverse effects will occur, and the consequences of very long-term treatment in humans are not known, and TGF-β blockade might become pathogenic above a certain threshold.

DRUGS WITH MULTIPLE ACTIONS THAT INHIBIT TGF-β

Tranilast (*N*-[3,4-Dimethoxycinnamoyl]Anthranilic Acid)

This drug was developed by Kissei Pharma (Japan) and has been studied in several thousand patients for various indications.[110] It blocks mast cell degranulation and has been used clinically in Japan and South Korea for the treatment of allergic disorders (asthma, allergic rhinitis and atopic dermatitis).[110–114] In addition, it has potent antifibrotic effects,[110,115–117] and has been successfully used for the treatment of hypertrophic scars and keloids. It might also find applications in renal disease and myocardial fibrosis.[111] Importantly, many years of clinical use have revealed that it is safe and well tolerated by most patients at doses of up to 600 mg/day for months.[110] Obviously, this represents a major advantage over other drugs that are in the early or mid-phase of development. In animals, the antifibrotic effects of tranilast have been demonstrated extensively in various disease models.[111,118–125] They are also apparent in a newly described *in vitro* model of fibrosis, designed for screening antifibrotic drugs.[126]

The mechanisms of tranilast's antifibrotic effects are not fully understood, but a major effect is the inhibition of both TGF-β expression and action.[115,123–139] In cell culture assays, tranilast inhibited both TGF-β secretion and TGF-β receptor expression.[12,129] Furthermore, it inhibited phosphorylation of Smad2[125] and ERK,[131] suggesting that it impedes both Smad-dependent and -independent TGF-β signaling pathways. Gilbert and co-workers[125,133,136] examined the effects of tranilast on TGF-β-induced matrix synthesis, and found that it was suppressed both *in vitro* and *in vivo*. These investigators assessed TGF-β expression in target tissues, as wells as Smad phosphorylation, by examining the expression of phosphorylated Smad2 with an mAb that detects only the phosphorylated form. Their studies showed dramatic attenuation of both molecules with tranilast treatment.

However, this inhibitory effect is not specific, as tranilast also inhibits the production of other cytokines, including IL-6, IL-12, IFN-γ, and monocyte chemoattractant protein-1.[123,132,140,141] As such, it exerts at least mild immunosuppressive effects. For instance, we recently found that it inhibits production of IL-17 by lymphocytes and tumor cells (unpublished observations). This cytokine has been linked to both autoimmunity and tumor progression.[10] Interestingly, tranilast was protective against experimental autoimmune encephalomyelitis (EAE),[140] where IL-17 is thought to play an important pathogenic role. In addition, tranilast strongly inhibits PGE2 production[132] and antagonizes the effects of VEGF;[142] two mediators that are involved in immunity, wound healing and cancer progression.

In recent years, on the basis of successful preclinical studies, tranilast was investigated for the prevention of restenosis after percutaneous transluminal coronary revascularization (PRESTO clinical trial), but was not found effective in that application. However, there is early evidence that it might be effective against intestinal stricture progression in Crohn's disease[143] (which is partly TGF-β dependent). If confirmed, this would be of considerable interest because more specific inhibitory drugs, such as ALK5 blockers, might enhance the inflammatory component of the disease by removing TGF-β's suppressive effect. In contrast, tranilast appears to be able to inhibit TGF-β while maintaining an immunosuppressed environment, presumably through the co-inhibition of inflammatory mediators.

Tranilast has notable antitumor effects, but the mechanisms are not fully understood and might involve several factors. *In vitro*, it exerts antiproliferative effects on fibroblasts, vascular smooth muscle cells, lymphocytes and tumor cells[131,140,144–146,161] (our unpublished observations). It inhibits the proliferation of uterine leiomyoma cells *in vitro* through G1 arrest associated with the induction of the cyclin-dependent kinase inhibitor p21 and the tumor suppressor p53.[145,146] In addition, as noted above, it inhibits PGE2 and VEGF effects that are relevant to tumor progression.

In tumor cell cultures of various origins (eg, breast, stomach, lung, pancreas, CNS/glioma) it inhibited TGF-β (all isoforms examined) and antagonized TGF-β-mediated effects on cell migration and proliferation at non-cytotoxic concentrations. *In vivo*, it inhibited the growth of 9L glioma cells and reduced the expression of TGF-β2.[131] The proliferation of the human schirrhous gastric cancer cell line OCUM-2M was inhibited *in vivo* by tranilast alone, or in combination with cisplatin.[162] Interestingly, the invasive ability of OCUM-2D cells was significantly increased by co-culturing with fibroblasts (NF-10 cells), which produce TGF-β1 and this effect was countered by tranilast.[134] Other investigators[163] reported that tranilast inhibited growth and lymph-node metastasis of the OSC-19 human squamous cell carcinoma cell line in nude mice. Fibrous tissue, microvessel density, and the PCNA labeling (proliferative) index of the tumors were significantly reduced. Similarly, tranilast exerted antitumor and antiangiogenic effects in a murine Lewis lung carcinoma model, and it potentiated the effects of cyclophosphamide, adriamycin and other anticancer drugs.[164]

INHIBITORS OF ANGII ACTIVITY

There are multiple interactions between the RAS and TGF-β pathways, some of which can be blocked with antihypertensive drugs.[53,147–149] Indeed, AngII increases TGF-β production by some cell types in the kidney, cardiovascular system and other tissues. It also enhances the expression of the TGF-β receptors through the MAPK pathway.[165] Moreover, AngII can activate the Smad signaling pathway independently of TGF-β.[53] Both AngII and TGF-β increase release of CTGF. As a result, increased levels of AngII, as in many diseases, can lead to fibrosis and various TGF-β-related pathology. In this respect, it should be noted that TGF-β participates in several cardiovascular conditions including healing myocardial infarcts (MIs), coronary artery restenosis, cardiac hypertrophy, hypertrophic and dilated cardiomyopathies, and hypertension. It also has a complex role in

atherosclerosis, which is still being elucidated. Similarly, TGF-β can be linked to many renal diseases, where there is either glomerulopathy or interstitial fibrosis.

Drugs that inhibit the RAS include angiotensin-converting enzyme (ACE) inhibitors and antagonists of the AngII type 1 receptor (AT1). They are safe drugs commonly used to treat hypertension, but clearly have activities beyond their antihypertensive effects, including inhibition of TGF-β. For instance, Losartan, an AngII receptor blocker, reduced TGF-β production and ameliorated disease in a rat model of bleomycin-induced pulmonary fibrosis.[150] Similarly, it inhibited TGF-β production in mice with either fibrilin-1 or dystrophin deficiency, and this improved muscle regeneration in these disease models.[151] Interestingly, some investigators have shown that high glucose levels stimulate TSP-1-dependent TGF-β activation in glomerular mesangial cells.[152] TSP-1, as mentioned previously, activates latent TGF-β. They found that AngII also upregulated TSP-1 production and TSP-1-dependent TGF-β activation by mesangial cells and, furthermore, that rat cardiac fibroblasts responded similarly. These AngII effects were blocked by Losartan. The glucose and AngII stimulation of TGF-β activation appear to be synergistic, and this is clearly relevant to both diabetes and hypertension.

In the remnant kidney model, characterized by renal TGF-β production and associated glomerulosclerosis and interstitial fibrosis, both tranilast and the ACE inhibitor perindopril were protective.[139] Both drugs were capable of inhibiting TGF-β activity as manifested by reduced nuclear phosphorylated Smad2. The combination of the two drugs was more effective than either alone, and perindopril provided the additional benefit of blood pressure reduction. Of note, the ACE inhibitor was at least as effective as tranilast in suppressing the TGF-β response. However, from the current literature, it does not appear that inhibitors of the RAS system are as effective as tranilast in mediating anti-inflammatory, antiproliferative and anticancer effects.

Clinically, treatment with ACE inhibitors or AngII receptor antagonists appears to alleviate TGF-β-dependent pathology in several renal and cardiovascular diseases.[53,149] It seems likely that they would also be of benefit in cirrhosis and other diseases characterized by fibrosis. In view of their favorable safety profile, they might be appropriate agents to treat these conditions. However, at least in renal disease, recent studies suggest that these drugs are more effective in rodent disease models than in humans.[153] Interestingly, statins have anti-fibrotic and anti-inflammatory activity, and may interfere with some TGF-β-mediated effects (Table 2). However, the mechanism of action and clinical significance of statins in the context of TGF-β-induced disease have not been extensively characterized.

TGF-β DELIVERY FOR THERAPEUTIC PURPOSES

In the previous sections the author has concentrated mostly on the negative effects of TGF-β, but this cytokine has several positive effects that might also be amenable to therapy. This includes autoimmune diseases, as we have previously reviewed,[24,166] wound healing and cardiac remodeling after ischemic injury.

In preclinical models of autoimmune disease, we and others found that TGF-β gene therapy, or in some cases protein therapy, is beneficial in autoimmune (type 1) diabetes (T1D), EAE, inflammatory bowel disease and various types of arthritis.[24,166] It should be noted that both active and latent TGF-β were effective but, in the case of protein therapy, relatively large amounts had to be administered. The use of gene therapy approaches, such as intramuscular delivery of expression plasmids, or administration of various viral vectors, allows relatively long-term expression of the cytokine at therapeutic levels.[166] Gene transfer with plasmids is greatly improved by *in vivo* electroporation and raises fewer safety concerns than viral therapy,[167] but these gene therapeutic approaches are not approved for clinical use. Furthermore, there are a number of obstacles to systemic TGF-β therapy, and its pleiotropic effects raise many safety issues. In animals, consistent with the known activities of TGF-β1, chronic administration (or transgenic overexpression) has led to interstitial fibrosis, glomerulosclerosis, hepatic fibrosis, cardiac disease and lesions in several other target tissues.[1,168] Another caveat, at least in the area of autoimmune diseases, is the recent realization that, in conjunction with IL-6, TGF-β promotes the differentiation of inflammatory Th17 cells. This is highly context dependent (influenced by many cytokines), and either beneficial or detrimental effects might be observed depending on the type of autoimmune disease, or the stage of disease at the time of treatment.

In clinical trials, systemic therapy has been associated with nephrotoxicity, anemia and other adverse effects (reviewed in Flanders and Roberts[1]). For this reason, TGF-β therapy was abandoned by most pharmaceutical companies before the year 2000. Indeed, toxicity and pleiotropic effects are a general limitation of therapy with other cytokines as well. In view of this, local therapies appear more promising, and the ability of TGF-β to promote healing is of great interest. For instance, in preclinical trials of diabetic wound healing, this has been achieved by local electroporation-enhanced TGF-β gene transfer.[169] Whether these gene therapy approaches can be transferred to the clinic remains to be determined. As a caveat, a clinical trial of topical TGF-β3 protein therapy of skin ulcers showed only modest benefits,[170] and clinical experience with local TGF-β therapies remains very limited. Moreover, local therapies carry the risk of excessive fibrosis and scar formation.

Another possible application is in the area of cardiovascular disease. TGF-β regulates many of the events of the healing MI. This ranges from the early inflammatory response, to the late fibrotic response and the associated myocardial remodeling. There is also evidence that TGF-β can protect cardiomyocytes from ischemic injury, as recently reviewed.[148,171] However, limiting therapeutic intervention,

there is the realization that TGF-β has both positive and negative effects on the healing heart,[102,148] and is a contributor to cardiac hypertrophy and vascular disease. It can be beneficial in atherosclerosis by stabilizing plaques,[3,102,172] at least in animal models, but it also promotes vascular restenosis. Therefore, the appropriate timing of either TGF-β or anti-TGF-β therapy would be critical, and much more research is required in this area.

Returning to autoimmune diseases, an alternative approach to TGF-β therapy is the induction of Tr cells that produce this cytokine. Experimentally, this has been achieved by induction of oral tolerance,[48,49] but in human disease this approach has met with limited success. However, there is early evidence that some drugs can induce differentiation of TGF-β-producing Tr cells, and this might be an avenue for therapy. Furthermore, we have shown that DNA vaccination can be applied to the generation of these Tr cells.[173] In our experiments, an autoantigen gene was co-delivered with a selective CTLA-4 ligand (mutated B7-1). This led to the generation of Foxp3$^+$ Tr cells that appear to act by producing TGF-β. CTLA-4 might act by priming the T cell for responsiveness to TGF-β and subsequent Foxp3 induction. Other vaccination approaches to induce TGF-β-producing cells are also feasible.[174] In addition, the systemic administration of CD3 mAb leads to the generation of TGF-β-producing Tr cells and protects mice against autoimmune diabetes, and this might be clinically applicable.[175] In all these cases, the advantage is that Tr cells are generally antigen specific, and can home in to the target tissues and, presumably, exert a local effect rather than a systemic effect.

CONCLUSIONS AND FUTURE PROSPECTS

In this review, the author has demonstrated the pleiotropic activity of TGF-β in a number of physiological and pathological processes. This cytokine contributes importantly to healing, immunoregulation and cancer progression. These effects are highly context dependent, and can be either beneficial or detrimental. However, a large number of diseases (including cancer) are characterized, at least at some phases, by TGF-β overproduction and are amenable to anti-TGF-β therapy. This can be accomplished by several methods, including antibodies, soluble receptors, receptor kinase antagonist drugs, antisense ODNs and a number of less specific drugs that are in widespread or limited clinical use, such as AngII antagonists and tranilast.

In view of TGF-β's numerous functions, and potent anti-inflammatory effects, it might be assumed that its neutralization would be detrimental, or even fatal, but this has not proven to be the case. Indeed, relatively mild toxicity has been noted, but some adverse effects have been reported, and long-term safety remains to be established. Notably, AngII antagonists have few adverse effects, but the degree to which they inhibit TGF-β in humans requires further analysis. On the other hand, the direct administration of TGF-β for therapeutic purposes is more limited. This is due to toxicity and safety concerns, but local administration appears feasible, especially to promote wound healing.

Finally, the immunomodulatory effects of this cytokine are critically important, and the realization that it plays a key role in Tr-cell differentiation and survival is of major clinical interest. Drug therapy or vaccination therapy altering TGF-β production and Tr differentiation might be applied to the treatment of autoimmune diseases, transplant rejection and various inflammatory conditions. However, in this case as well, TGF-β's dual personality raises the concern that it could also induce the differentiation of inflammatory Th17 T cells, and actually aggravate some autoimmune/inflammatory diseases. Undoubtedly, future studies will yield valuable data about all these therapies.

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