

EDITORIAL

Transforming growth factor- β_1 : its anti-inflammatory and pro-fibrotic effects

Introduction

The TGF- β s are members of an essential, multifunctional family of cytokines. These molecules have pivotal roles to play in cell growth and development (at both embryonic and adult stages), during inflammation and the maintenance of host resistance mechanisms and within remodelling and repair processes such as angiogenesis and regeneration.

Of the three mammalian isoforms of TGF- β (TGF- β_1 , TGF- β_2 and TGF- β_3), TGF- β_1 is the only member where the homozygous negative mouse can survive following the neonatal period. TGF- β_2 and 3 knockout mice die rapidly postnatally [1,2]. The phenotype of TGF- $\beta^{-/-}$ mice initially appears quite normal; however, within a couple of weeks after birth the animals develop a severe wasting disease and die within a month [3,4]. The cause of death has been shown to be massive inflammatory lesions in multiple organs, which results in lethal cardiopulmonary failure [5].

TGF- β_1 , TGF- β_2 and TGF- β_3 all arise from precursor proteins, which are processed to produce a latent TGF- β complex that is secreted by the cell [6–8]. Either before or after secretion, it can associate with other proteins to form higher molecular weight complexes [9,10]. The TGF- β receptors, of which at least six have been described [11], either mediate an intracellular signal and/or present TGF- β s to signalling receptors [12]. For TGF- β to be able to signal through these receptors the latent complex must first be activated. This process of activation is still not well understood; however, proteins such as plasmin, cathepsin and thrombospondin-1 have all been shown to activate latent TGF- β_1 complexes [13–15]. The signal pathways activated by TGF- β are transduced by a series of SMAD proteins [11,16]. This pathway is very unusual in that there is only one amplification step between the signal being received and the target gene being activated in the nucleus [17].

TGF- β_1 and the immune system

TGF- β_1 has a unique and essential function in regulating immune responses, most dramatically seen in the severe inflammatory phenotype of the TGF- β_1 knockout mouse. The wide-ranging effects of TGF- β on cells of the immune system can seem confusing and contradictory (see Table 1). This cytokine can stimulate cellular growth, differentiation, migration and cytokine production but can also inhibit these very same processes. TGF- β inhibition or stimulation can depend on the cell type or in some cases even the level of differentiation of a particular cell.

TGF- β was originally identified as a growth factor for mesenchymal cells [18]. It also has stimulatory effects on many other cells. Early in an immune response TGF- β is released

from local platelet stores. At this stage of the response it mediates leucocyte recruitment and activation by increasing adhesion molecules, generating chemoattractant gradients and inducing pro-inflammatory cytokines including TGF- β itself [19–21]. TGF- β has been found to have a chemoattractant effect on monocytes, macrophages, fibroblasts, neutrophils and mast cells [22]. When activated in the presence of TGF- β T cells show an increase in proliferation, IL-2 production and expression of α E β 7 and CD2 receptor, along with decreased CD11a expression. These effector T cells will also show a strongly enhanced capacity to respond to subsequent stimulation [23]. In some models B-cells too show some signs of activation following TGF- β stimulation. Snapper et al. [24] showed that the addition of anti-TGF- β blocking antibodies to LPS-activated cultures led to a significant decrease in levels of IgG1, IgG2a, IgG3 and IgE.

If TGF- β_1 is a potent initiator of the early immune response, why then does the TGF- β_1 knockout mouse suffer from an uncontrolled inflammatory condition? The inhibitory side to TGF- β_1 can help to explain this. Following an inflammatory response, TGF- β_1 also plays a part in down-regulating the inflammatory process by inhibiting the functions of activated cells and even promoting apoptosis [25,26]. TGF- β inhibits proliferation and cytokine production by naïve T cells and both Th1 and Th2 T cell clones, and appears to be responsible for part of the inhibitory effect of CTLA-4 T cell signalling [27]. It also acts as a chemo-attractant to bring macrophages into the area and the very ingestion of apoptotic cells by macrophages causes inhibition of pro-inflammatory cytokines through a mechanism involving TGF- β_1 as well as PGE₂ and PAF [28,29]. Resolution of inflammation, therefore, is also dependent on a TGF- β_1 -mediated mechanism. TGF- β is also needed as an inhibitor of immune responses for maintenance of immune homeostasis. Endogenous TGF- β is protective against both Th1 cell-mediated autoimmunity and allergic inflammation. This can be illustrated by the adverse effect of neutralizing anti-TGF- β antibodies on the course of experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA) [30,31]. TGF- β has also been implicated in the oral tolerance model [32]. These effects may be mediated by production of TGF- β by regulatory T cells *in vivo*, although most *in vitro* studies of CD4⁺ CD25⁺ regulatory T cells show that regulation is not reversed by blocking antibodies to TGF- β [33]. There are also reports that TGF- β has a role in the expansion of regulatory T cells from human peripheral blood [34].

As well as the stimulatory and inhibitory influences of TGF- β on the immune system, the TGF- β_1 knockout mouse has shown that TGF- β_1 has a critical role to play in shaping the repertoire of T cells through selection and self-tolerance. In the thymus of

Table 1. Examples of some of the effects of TGF- β on different cell types

Target cell type	Effect of TGF- β	Reference
Osteoclasts	Inhibits formation of osteoclasts and shortens life span of mature osteoclasts	47
Cultured vascular smooth muscle cells	Stimulates expression of thrombin receptors Induces cellular apoptosis	48
BCG-activated T cells	Down-regulates BCG induced cell growth and IFN- γ production	25
Priming CD4 ⁺ T cells	Down-regulation of α CD3/ α CD28 and antigen-specific 2° immune response	49
Mitogen activated PBMCs	Increase in apoptosis	26
Monocytes/fibroblasts/ neutrophils/mast cells	Chemotactic effect	22
Macrophages that have ingested apoptotic cells	Inhibition of pro-inflammatory cytokines. Increase in TGF- β , PAF and PE ₂	27
Naïve T cells activated in the presence of TGF- β	Increase in proliferation, IL-2, CD2R and aE β 7. Enhanced capacity to respond to subsequent stimulation	23

the knockout mouse, thymocytes are excessively sensitive to TCR-enforced lethality. This means that both positive and negative selection of thymocytes is disrupted, resulting in loss of self-reactive T cells into the periphery. These dangerous escapee T cells cause the autoimmune inflammation to start. Cells with engaged TCR are extremely sensitive to apoptosis and cell death. This should be a helpful process removing antigen-activated T cells and resolving inflammation, but in the absence of TGF- β_1 , macrophages don't inhibit pro-inflammatory cytokines and inflammation continues relentlessly (reviewed in Wahl et al. [35]). Looking at the effects of TGF- β_1 on different cell types at different stages of activation, as shown in Table 1, does at first appear confusing and contradictory, but as more pieces of the puzzle are put together in context the picture becomes a lot clearer.

TGF- β_1 and tissue repair

TGF- β is a cytokine vital to tissue repair. It is thought to be the active wound-healing ingredient in saliva; the reason why animals lick their wounds and maggots promote fast wound healing. It has also been implicated, however, in scarring and causing more serious disease by promoting tissue damage. We have already heard how TGF- β_1 could potentially clean up a wound site, first by attracting and activating monocytes and leucocytes to fight infection, then promoting apoptosis of these same cells and clearance by macrophages producing anti-inflammatory cytokines. Next, it would induce angiogenesis [36] and increase deposition of extracellular matrix [37] to close the wound. TGF- β_1 also blocks matrix degradation by decreasing the synthesis of proteases and increasing the level of protease inhibitors [38,39]. In many different disease states caused by inflammatory mediated tissue damage, elevated TGF- β levels have been found at the affected site, for example kidney disease [40], scarring of the skin [41] and asthma [42]. The problem may occur as a result of the fact that TGF- β_1 induces its own production through a positive feedback loop. If this isn't properly regulated then fibrosis will occur.

The different faces of TGF- β_1

TGF- β_1 does have many tantalizing properties that could be of great therapeutic benefit. It is a potent immunosuppressive agent so it could be used to treat autoimmune diseases and

other chronic inflammatory conditions such as atopic dermatitis (as featured in this month's journal by Sumiyoshi et al. [43]). These authors used exogenous TGF- β in a mouse strain that develops spontaneous skin lesions with some similarities to human atopic dermatitis. In this model TGF- β down-regulated skin lesions and mononuclear cell and eosinophil infiltration and reduced total IgE. However, cytokine production by splenocytes showed reduction in IFN- γ but not Th2 cytokines. As TGF- β can down-regulate both Th1 and Th2 responses this may be relevant here: in human atopic dermatitis a mixed Th1/Th2 picture is also seen. It is of note that the Tr1 regulatory T cell subtype could reduce airway inflammation and eosinophilia in a mouse model, as well as the Th1-predominant colitis model. The regulatory activity of these cells appears to involve both TGF- β and IL-10 [44]. TGF- β regulatory activity may thus be of interest in both allergic and autoimmune diseases.

TGF- β promotes wound healing and could also be a possible cancer therapy. Inhibition of TGF- β could be useful in diseases where fibrosis occurs [41]. Such a stimulatory role is noted for TGF- β in a second paper in this edition by Wright et al. [45], where TGF- β is shown to promote mouse mucosal mast cell differentiation from bone marrow cells, and particularly expression of mast cell proteases. Whether this has a role in mast cell-induced fibrosis remains to be established.

A potential problem, however, is that it is difficult to dissect the 'wanted' properties from the 'unwanted'. For example, using TGF- β as an immunosuppressive agent could be very effective, but would there be side-effects such as fibrosis?

How is it possible for one molecule to have so many different effects within the same organism? There are many different aspects of TGF- β_1 that could account for its diverse range of functions. TGF- β_1 goes through a complex synthesis and secretion process. Whilst still within the cell the pro-TGF- β monomer dimerizes and binds to the latency-associated peptide to form a complex, it may also bind to a 135-kDa latency-binding peptide [12]. The latent TGF- β complex is then released from the cell and can be activated. The bioactive TGF- β molecule is usually either a homo- or heterodimer of 25kDa. It is, however, known that bioactive TGF- β molecules of atypical mass exist. This could be due to different genes, processing by different enzymes, or TGF- β complexing with additional proteins. Any of these could cause a conformational change in the TGF- β molecule that could equate to an altered function of the protein. A particular active site could be masked or another exposed, perhaps allowing the molecule to bind to a receptor that is restricted to a specific target cell. Work done on these atypical,

bioactive TGF- β s may yield valuable information on the structure/function relationship of TGF- β . The concentration of TGF- β and other cytokines in the *milieu* that could enhance or inhibit function may also have an effect. These concentrations could be altered due to changes in the removal rate by a variety of degradation enzymes. The target cell type also makes a big difference as to the outcome of TGF- β binding. This may be because some cells have specific TGF- β receptors and perhaps at different activation states they may express different levels of the receptor. Receptors of different types may have altered signalling pathways that activate other genes and cause varying effects.

The diverse signalling pathways of the TGF- β family have recently been investigated using SMAD knockout mice (reviewed in Weinstein et al. [46]). The work has shown that different SMAD proteins are required for the functions of TGF- β . For example, SMAD 2 and 4 are required for gastrulation, SMAD 5 for angiogenesis and SMAD 3 for establishment of the mucosal immune response. The SMAD 3 knockout mouse has a very interesting phenotype, as certain cellular responses to TGF- β are selectively lost. One outcome being that wound healing processes are accelerated, wounds that take 5 days to heal in wild-type mice heal in 2 days in these mice. This would seem to be a strange phenotype as TGF- β has been found to enhance wound healing.

The cell types that are involved in the wound healing process are fibroblasts, which are stimulated to produce extracellular matrix by TGF- β_1 , monocytes, which are strongly chemoattracted by TGF- β_1 , and keratinocytes, which conversely are strongly inhibited by TGF- β_1 . This inhibition of keratinocyte proliferation seems very odd for a molecule that enhances wound healing, but the SMAD 3 deficient mouse might have begun to explain things. The SMAD 3 arm of the signalling pathway seems to inhibit the rate of wound healing by inhibiting keratinocyte proliferation, whereas another SMAD must promote the production of extracellular matrix by fibroblasts. The inhibitory SMAD 3 arm may slow down the process of healing to make sure that the wound heals properly and is strong. Cells that don't contain SMAD 3, however, will heal more quickly. These studies show that it might soon be possible to utilize one function of TGF- β_1 without compromising any of its other many effects throughout the body, through the local manipulation of SMAD proteins.

These studies hold the potential for dissecting the immunosuppressive effects of T cell-derived TGF- β from profibrotic or airway remodelling effects of TGF- β from structural cells such as airway epithelial cells. In the context of allergic disease (and particularly asthma) we may thus be able to resolve the paradox of wanting to exploit the regulatory effects of TGF- β whilst preventing the remodelling promoting activity.

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