Chapter 12
TGF-β and Inhibitory Smads in Inflammation

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Abstract TGF-β is a multifunctional cytokine involved in diverse cellular functions, including cell growth, apoptosis, and immune responses, although its effect is likely to be dependent on cell context. Among a variety of cellular functions exerted by TGF-β, recent advances emphasize the importance of TGF-β and its signaling pathway in innate immunity and adaptive immunity. The typical examples of immune regulations by TGF-β include suppression of toll-like receptor (TLR) signaling which recognizes the invading pathogens and T cell differentiations such as Th17 and Treg. In particular, much attention has been paid to anti-inflammatory function of TGF-β, which is mediated by the inhibitory Smads, Smad6 and Smad7. In this review, we mainly discuss the anti-inflammatory role of TGF-β suppressing inflammatory responses and the underlying mechanism mediated by the inhibitory Smads.

Keywords Inflammation • Inhibitory Smads • Innate immune responses • Smad6 • Smad7

12.1 Introduction

Although extensive studies have been performed to reveal essential roles of TGF-β and the related molecular mechanism during the past 25 years (Blobe et al. 2000; Derynck and Zhang 2003; Heldin et al. 1997; Letterio and Roberts 1997; Massague 1998; Miyazono et al. 2000), the functions of TGF-β and its signaling pathway are

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very complicated and controversial. The major reason is that TGF-β signaling pathways are involved in most cellular functions showing cell context-dependent fashion and forming highly intricate signaling networks through cross talking with other cellular signaling pathways.

There are three TGF-β members (TGF-β1, TGF-β2, and TGF-β3) present in mammals. Among them, TGF-β1 is the major form found in the immune system. TGF-β signaling pathways are mainly divided into canonical and noncanonical pathways (Derynck and Zhang 2003; Mu et al. 2012). TGF-β canonical pathway is mediated by heterodimeric receptors with serine/threonine receptor kinases and cytoplasmic proteins called Smads. Upon binding to TGF-β, type II (TβRII) and type I (TβRI) receptors initiate the signaling process with their intrinsic kinase activities, and then the activated type I receptor transmits intracellular signals through the phosphorylation of receptor-activated Smad proteins (R-Smads), Smad2 and Smad3. The phosphorylated R-Smads form heteromeric complexes with common partner Smad4 (Co-Smad) and translocate into the nucleus to modulate expressions of diverse target genes (Derynck and Zhang 2003; Massague and Wotton 2000; Miyazono et al. 2000). The more interesting finding is that this canonical TGF-β signaling has its own regulatory system, called inhibitory Smads (I-Smads). The I-Smads include Smad7 and Smad6 proteins (Hayashi et al. 1997; Imamura et al. 1997; Nakao et al. 1997). Smad7 is transcriptionally induced by TGF-β and acts as an intracellular antagonist through stable interaction of activated TβRI receptor, resulting in the formation of autoregulatory negative feedback loop of TGF-β signaling (Hayashi et al. 1997; Nakao et al. 1997). Smad6, another inhibitory Smad, is also induced by TGF-β in several cell lines such as mink lung epithelial (Mv1Lu) cells, human keratinocyte (HaCaT) cells, CMT-93 mouse intestinal epithelial cells, and mouse primary peritoneal macrophages (Afrakhte et al. 1998; Choi et al. 2006). It has been initially identified as an inhibitor of Smad2 and Smad1 phosphorylation by TGF-β superfamily (Imamura et al. 1997; Ishida et al. 2000), and it is likely to be an important regulator involved in TGF-β signaling (Afrakhte et al. 1998; Choi et al. 2006; Kleeff et al. 1999).

In contrast, TGF-β noncanonical signaling is independent of the activations of R-Smads, and TGF-β utilizes a variety of cellular signaling pathways such as p38 and JNK mitogen-activated protein kinases (MAPK), PI3 kinase, and TRAF6-TAK1 pathways (Mu et al. 2012; Zhang 2009). Therefore, TGF-β noncanonical pathways complicate the understanding of the precise molecular mechanisms underlying TGF-β-mediated cellular functions.

Inflammation is defined as a protective response to eliminate detrimental stimuli and to repair damaged tissue (Medzhitov 2008). Inflammatory stimuli include a variety of factors such as microbial infections, necrotic tissue injuries, and cytokines (Takeuchi and Akira 2010). In terms of tissue homeostasis, inflammation should be resolved in a timely manner and damaged tissues should restore their normal structural and functional state. If inflammatory responses is not resolved, persistent inflammation may become a key factor causing chronic inflammatory diseases such as cancer, diabetes, sepsis, and atherosclerosis (Nathan and Ding 2010).

The innate immune system mediates inflammatory responses induced by microbial infection or tissue damage (Akira et al. 2006; Beutler et al. 2006; Takeuchi and Akira 2010). The innate immune responses are initiated by pattern recognition receptors (PRRs) which recognize pathogen-associated molecular patterns (PAMP) or damage-associated molecular patterns (DAMPs) (Takeuchi and Akira 2010). Among PRRs, toll-like receptors (TLRs) recognize PAMPs and transmit signals to downstream proteins through adaptor MyD88-dependent or MyD88-independent pathways, resulting in the activation of NF-κB or IRF3 protein to increase expression of pro-inflammatory cytokines and interferon-β1 (O'Neill and Bowie 2007; Takeuchi and Akira 2010).

Therefore, to maintain tissue homeostasis, TLR activation causing the inflammatory responses must be regulated, and aberrant regulation of TLR signaling is known to induce hyper-inflammatory diseases (Cook et al. 2004). Interestingly, anti-inflammatory activities of TGF-β cytokine have been reported in knockout mice studies, but the molecular mechanisms as to how TGF-β signaling is interconnected with innate immune responses and inflammations have not been addressed as much as other mechanisms mediated by TGF-β such as cell growth and apoptosis. Therefore, we review recent progresses on the TGF-β signaling pathway regulating inflammation and innate immune responses.

12.2 TGF-β and Inflammation

12.2.1 Effects of TGF-β Signaling Components on Inflammation

Previous studies on animal models of TGF-β signaling deficiency have clearly substantiated the importance of TGF-β signaling for immune functions and inflammation. The TGF-β1-null mutation in mice triggered an autoimmune inflammatory condition involving nuclear autoantibodies and autoreactive T cells. These mice had excessive inflammatory responses and developed a multifocal inflammatory disease resulting in cardiopulmonary complications that were ultimately lethal (Kulkarni et al. 1995; Shull et al. 1992). Histopathological analysis of these mice revealed massive infiltration of lymphocytes and macrophages in many organs, especially in the heart and lungs. In the spleen and the lymph nodes, inflammatory lesions included proliferation of immunoblasts and lymphoblasts in B and T cell zones. Inflammatory lesions were also seen in the pancreas, salivary glands, colon, and stomach of some of the TGF-β1(−/−) mice. In addition, conditional KO mice of TβRII gene, when it was disrupted by poly I:C induction in hematopoietic cells, showed a lethal inflammatory disease affecting multiple organs (Leveen et al. 2002). Bone marrow from conditional knockout mice of TβRII transferred to normal recipient mice caused a similar lethal inflammation, regardless of whether induction of TGF-β receptor deficiency occurred in donor animals before or in recipient animals

after transplantation. These results demonstrate that TGF-β signaling deficiency within cells of hematopoietic origin is sufficient enough to cause a lethal inflammatory disorder in mice. Another kidney-specific conditional KO mice of TβRII gene enhanced NF-κB signaling and renal inflammation including IL-1β and TNF-α in the model of unilateral urethral obstructive (UOO) nephropathy (Meng et al. 2012a). The important role of TGF-β in immune homeostasis has been previously suggested by the observation of multifocal immune-mediated inflammation in CD4-dnTGF-βRII transgenic mice similar to that of TGF-β1 KO mice. The CD4-dnTGF-βRII transgenic mice developed autoimmune disease characterized by inflammatory infiltration in several organs and the presence of circulating autoimmune antibodies, indicating that T cell homeostasis and prevention of inflammatory infiltration require TGF-β signaling in T cells (Gorelik and Flavell 2000). Among Smad proteins, Smad3 KO mice displayed impaired mucosal immunity and reduced T cell responsiveness (Ashcroft et al. 1999; Yang et al. 1999). Smad3-deficient mice were normal during embryonic and early postnatal development. After weaning, Smad3 KO mice invariably developed an illness associated with progressive leukocytosis, periodontitis, gastritis, colitis, and chronic infection with abscess formation adjacent to mucosal surfaces, suggesting that Smad3 has an important role in TGF-β-mediated regulation of T cell activation and mucosal immunity. The important role of Smad4 in inflammation has recently been suggested in a mouse model of unilateral urethral obstruction using conditional Smad4 knockout mice and in isolated Smad4 mutant macrophages and fibroblasts (Meng et al. 2012b). Disruption of Smad4 significantly enhanced renal inflammation as evidenced by a greater CD45(+) leukocyte and F4/80(+) macrophage infiltration and upregulation of IL-1β, TNF-α, MCP-1, and ICAM-1 in the obstructed kidney and in IL-1β-stimulated macrophages. These results suggest that Smad4 may also be a key regulator for the diverse roles of TGF-β1 in inflammation. The studies of animal models described in this review demonstrate the significant role of TGF-β in the regulation of T cell homeostasis and prevention of immune inflammation in many organs. These mice provide good models to study the pathogenic mechanisms in animal deficient of TGF-β signaling and to elucidate the specific cellular and molecular mechanisms of TGF-β that maintain homeostasis within the immune system.

Evidence of the anti-inflammatory role of TGF-β in human was shown in the study using biopsy specimens. Blockade of TGF-β in normal intestinal biopsies grown ex vivo and lamina propria mononuclear cells (LPMCs) downregulated T cell apoptosis and induced a significant increase in pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-12, IL-6, IL-18, and IL-17, supporting an anti-inflammatory role of TGF-β in dampening T cell-mediated tissue-damaging responses in the human gut (Di Sabatino et al. 2008). On the contrary, the opposite results were observed in colonic biopsies of patients with ulcerative colitis (UC) or Crohn's disease (CD) (Babyatsky et al. 1996). The area of active inflammation in UC or CD showed an increased level of TGF-β expression. Surprisingly, the increased level of TGF-β in UC or CD did not exert an anti-inflammatory function (Monteleone et al. 2001). The reason was due to the overexpression of Smad7, an inhibitory Smad, in intestinal mucosa derived from active CD and most UC patients

(Monteleone et al. 2001). The overexpressed Smad7 blocked Smad3 activation through interfering with TGF-β type I receptor in these inflammatory diseases. Even though a number of studies are still performed to understand biological functions of TGF-β, these results basically suggest that in vivo functions of TGF-β signaling pathway are critically connected into immunological responses including innate immunity and adaptive immunity.

12.2.2 Anti-Inflammatory Role of TGF-β Signaling Pathway Suppressing Innate Immune Responses

TLRs have been known to be an important defense system responsible for invading pathogens. However, the molecular mechanism behind how their signaling cascades are regulated by anti-inflammatory cytokine TGF-β has recently been demonstrated in several reports. The initial report about an anti-inflammatory role of TGF-β suppressing TLR4 signaling pathway stated that TGF-β inhibited MyD88-dependent TLR4 signaling through decreasing the MyD88 protein levels in dose- and time-dependent manner (Naiki et al. 2005). However, the molecular mechanism behind the polyubiquitination and subsequent degradation of MyD88 by TGF-β was not clearly demonstrated in this report. Recently, the inhibitory Smad6 protein was shown to be responsible for the polyubiquitination and selective degradation of MyD88 through the recruitment of E3 ubiquitin ligases Smurf proteins (Fig. 12.1a) (Lee et al. 2011). These observations suggest that MyD88-dependent TLR signaling pathways such as TLR4 and TLR2 are negatively regulated by the Smad6-Smurf pathway when treated with TGF-β. The more interesting finding was that another inhibitory Smad, Smad7, is not involved in MyD88 degradation, indicating that the two inhibitory Smads, Smad6 and Smad7, have distinct roles as anti-inflammatory mediators (Lee et al. 2011). However, Smad6 is likely to be targeting another adaptor protein Pellino-1 when suppressing TLR4 signaling (Choi et al. 2006). TGF-β-induced Smad6 protein binds to Pellino-1, an adaptor protein binding to IRAK1 (Grosshans et al. 1999; Jiang et al. 2003; Moynagh 2009) and in turn disrupts IRAK1-mediated signaling complex in TLR4 signaling (Fig. 12.1a). The disruption of IRAK1-mediated signaling complexes through sequestering Pellino-1 by Smad6 resulted in the inhibition of NF-κB-mediated pro-inflammatory gene expressions (Choi et al. 2006). These findings strongly suggest that Smad6 is a pivotal component of TGF-β-mediated anti-inflammatory network and targets MyD88-dependent and Pellino-1-mediated TLR signaling. Interestingly, recent reports showed that Pellino-1 has additional binding partners in TLR signaling as well as IRAK1. Pellino-1 binds to TRIF-associated RIP1 protein (Chang et al. 2009). Another report indicated that Pellino-1 interacted with the IKKε/TBK1 complex which phosphorylates IRF3 (Smith et al. 2011). These findings suggest that TGF-β-induced Smad6 disrupts the RIP1- and IKKε/TBK1-mediated signaling complexes and subsequently inhibits MyD88-independent TLR3 signaling pathway.

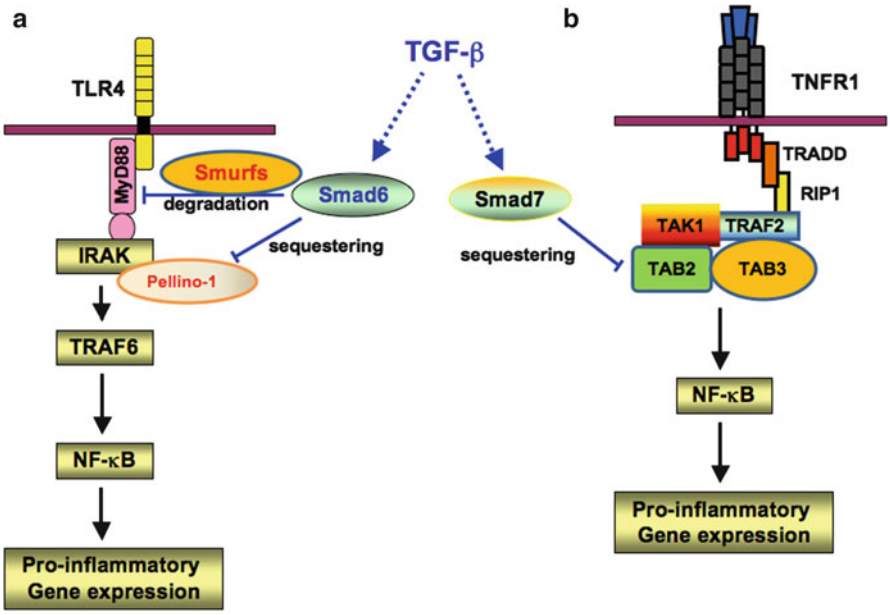

Fig. 12.1 Roles of the inhibitory Smads as mediators of anti-inflammatory TGF-β signaling. (**a**) TGF-β-induced Smad6 selectively degrades MyD88 protein in TLR4 signaling through recruiting E3 ubiquitin ligase Smurf proteins and also disrupts IRAK1-mediated signaling complex through sequestering the Pellino-1 protein. (**b**) TGF-β-induced Smad7 protein binds to TAB2 and TAB3 and subsequently suppresses TNF-α signaling through blocking the recruitment of the kinase TAK1 to the TRAF2 protein

In the case of Smad7, this molecule has been demonstrated to block pro-inflammatory TNF-α signaling (Hong et al. 2007). TGF-β-induced Smad7 interacts with the adaptors TAB2 and TAB3, and this interaction leads to the inhibition of TNF-α-induced NF-κB activity by blocking TAK1-associating proteins, TAB2 and TAB3, from forming a complex with TRAF2 (Fig. 12.1b) (Hong et al. 2007). Furthermore, Smad7 transgene expression in mouse skin under control of the keratin 5 promoter (K5-Smad7 mice) markedly suppressed inflammation and NF-κB nuclear translocation (Hong et al. 2007), suggesting that Smad7 is a critical mediator of the TGF-β pathway that blocks pro-inflammatory TNF-α signaling. Interestingly, Smad6 was not involved in TGF-β-mediated suppression of TNF-α signaling. TGF-β did not inhibit TNF-α-induced IL-6 expression in Smad7 knockdown primary peritoneal macrophages, whereas TGF-β still inhibited TNF-α-induced IL-6 expression in Smad6 knockdown primary peritoneal macrophages (Hong et al. 2007). Smad6 did not interact with TAB2. The Smad7 amino acids that are critical for TAB2-/TAB3-Smad7 interaction are not well conserved in Smad6. These findings strongly imply that Smad6 and Smad7 are differentially responsible for mediating anti-inflammatory TGF-β activity.

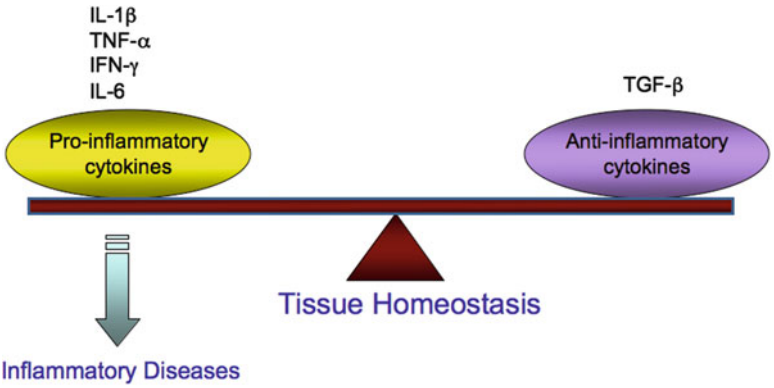

Fig. 12.2 Homeostatic balance between pro-inflammatory signals and anti-inflammatory TGF-β signaling under normal physiological conditions. The disruption of homeostatic balance between pro-inflammatory and anti-inflammatory signals, caused by excessive pro-inflammatory signals or abnormal reduction of anti-inflammatory signals, may be an important reason leading to the inflammatory diseases

Based on these findings, it is obvious that Smad6 and Smad7 play important roles in suppressing TLR4 signaling and pro-inflammatory TNF-α signaling, respectively. Although the TGF-β signaling pathway has been extensively studied, the physiological role of Smad7 and Smad6 requires further studies. The generation of Smad7 conditional knockout mice has been recently reported (Tojo et al. 2012); however, cell type-specific Smad6 conditional knockout mice are still unavailable. In vivo studies of these mice will elucidate how these molecules mediate TGF-β anti-inflammatory signaling.

12.2.3 The Inhibitory Smads as Cross Talkers of Intracellular Signaling Pathways

Recent reports suggest that inhibitory Smads may act as adaptors to facilitate cross talk between TGF-β signaling and pro-inflammatory signaling pathways. In this regard, Smad7 was extensively studied in the field of reciprocal regulation of TGF-β signaling and IFN-γ signaling. The pleiotropic cytokine IFN-γ suppressed TGF-β signaling through the increased expression of Smad7 by the activation of JAK-STAT1 pathway, which prevents the interaction of Smad3 with the TGF-β receptor and downregulates TGF-β signaling (Ulloa et al. 1999). Other groups reported another mechanism of IFN-γ-mediated suppression of TGF-β signaling (Ghosh et al. 2001). IFN-γ inhibited TGF-β signaling pathway through the sequestration of nuclear coactivator p300/CBP, which has been known to be associated with R-Smads (Ghosh et al. 2001). These results imply that the suppression of TGF-β signaling by IFN-γ will amplify the pro-inflammatory activity of IFN-γ under normal physiological conditions (Fig. 12.2).

In contrast, it has been reported that TGF-β inhibited IFN-γ-induced STAT1-dependent gene expression by enhancing STAT1-PIAS (a protein inhibitor of activated STAT1) interactions (Reardon and McKay 2007). However, these effects were shown in epithelial cells but not macrophages. Another group has shown that TGF-β inhibited IFN-γ-induced nitric oxide production in macrophages through the interaction of TGF-β type I receptor with IFN-γ receptor 1(IFNGR1) (Takaki et al. 2006). These results indicate that TGF-β suppresses the pro-inflammatory signal of IFN-γ to maximize its anti-inflammatory activity. Therefore, the anti-inflammatory and pro-inflammatory signals should be critically regulated to maintain tissue homeostasis (Fig. 12.2).

In addition, it was reported that TGF-β signaling is suppressed by NF-κB/RelA-dependent pathways (Bitzer et al. 2000). Activation of NF-κB/RelA by a variety of pathogenic and pro-inflammatory stimuli (TNF-α and IL-1β) increased transcription of the *Smad7* gene. This effect promoted the association of inhibitory Smad7 and type I TGF-β receptor upon TGF-β stimulation, leading to the suppression of TGF-β/Smad signaling. This gives further evidence that pro-inflammatory signals counteract anti-inflammatory signaling thresholds for TGF-β, dependent on physiological requirements to potentiate inflammatory responses. Similarly, a recent study supported the importance of a balance mechanism between TGF-β anti-inflammatory signal and pro-inflammatory signals. In this study, IL-1β or lipopolysaccharide (LPS) suppressed TGF-β-induced anti-inflammatory signaling in a NF-κB-independent manner through the interaction of TRAF6 with TGF-β type III receptor upon TGF-β stimulation, which then leads to pro-inflammatory factor-mediated attenuation of Smad2/3 phosphorylation (Lim et al. 2012). Although the antagonistic effects of IL-1β/LPS on TGF-β1 signaling may occur at multiple molecular levels, a fine modulation of the TGF-β receptors may critically affect cell fates by changing the kinetics of Smad2/3 phosphorylation. By this mechanism, IL-1β may potentiate inflammatory responses.

However, a recent report indicates that TRAF6-mediated polyubiquitination of transforming growth factor-β-associated kinase 1 (TAK1) activates NF-κB in HepG2 cells upon TGF-β1 treatment independent of p38 MAP kinase (Hamidi et al. 2012). This finding makes it difficult to understand the role of TGF-β1 in inflammatory responses. TAK1 is an important kinase in TGF-β noncanonical pathway, and TGF-β-induced Lys-63-linked polyubiquitination of TAK1 by TRAF6 is crucial for its activation and subsequent activation of p38 MAP kinase (Sorrentino et al. 2008). In this report, inflammatory stimuli, including IL-1β, TNF-α, and LPS, are able to induce TAK1 polyubiquitination and subsequent NF-κB activation in prostate cancer cells and RAW264.7 macrophages (Hamidi et al. 2012), suggesting that the increase of pro-inflammatory cytokines such as IL-6 may contribute to the formation of a tumor microenvironment. The interesting thing in this report is that TGF-β 1 also induces the same polyubiquitination of TAK1 and subsequent activation of NF-κB (Hamidi et al. 2012), suggesting that this phenomena may activate NF-κB survival signal in tumorigenesis. However, the authors did not show production of the pro-inflammatory cytokines in HepG2 cells upon TGF-β1 treatment as well as whether the activation of NF-κB upon TGF-β1 treatment is consistently observed in immune cells.

If TGF-β1-induced TAK1 and NF-κB activations are detected in immune cells such as macrophages and dendritic cells (DC), TGF-β1 is likely to have dual activities in inflammatory responses, as inflammatory or anti-inflammatory mediator and its activity might be differentially regulated according to cellular context or the related microenvironment.

On the other hand, the induction of Smad6 expression by pro-inflammatory signals is not reported, although Smad6 is transcriptionally induced by the anti-inflammatory cytokine TGF-β and responsible for TGF-β-mediated anti-inflammatory process (Afrakhte et al. 1998; Choi et al. 2006; Lee et al. 2011). A recent report showed that bacterial endotoxin LPS inhibits TGF-β-stimulated Smad6 expression in RAW264.7 macrophage cells (Kim and Kim 2011). The inhibition of TGF-β-induced Smad6 expression by LPS was mediated by phoshorylation of the Smad3 linker region through a TLR4-IRAK1-ERK1/2 pathway. The induction of Smad6 gene expression by pro-inflammatory signals has not been reported; however, we cannot exclude this possibility. It will be worth investigating whether pro-inflammatory signals potentiate inflammatory responses by stimulating Smad6 expression, which may counteract anti-inflammatory signaling thresholds for TGF-β/BMP.

12.2.4 A Role of TGF-β in T Cell Biology

TGF-β1 is one of the regulatory cytokines with pivotal functions in the control of inflammation. TGF-β1 directly targets T cells to ensure immune tolerance to self- and environmental antigens. Studies using animal models such as transgenic mice-expressing dominant-negative mutants of TβRII gene and T cell-specific TβRII KO mice indicated a principle role of TGF-β in T cell biology (Gorelik and Flavell 2000; Li et al. 2006; Lucas et al. 2000; Marie et al. 2006; Li and Flavell 2008). Generally, $CD4^+$ effector (helper) T cells are divided into two subsets, T helper type 1 (Th1) and T helper type 2 (Th2) cells, depending on the functions and cytokine production patterns. Th1 cells are characterized by their production of IFN-γ, a potent activator of cell-mediated immunity and thus responsible for removal of intracellular pathogens. Th2 cells that enhance eradication of parasitic infection are characterized by production of IL-4, IL-5, and IL-13. These helper T cells are critically regulated by regulatory T cells (Treg). Another subset of T helper cells is called Th17 cells which produce IL-17, a potent pro-inflammatory cytokine.

Recent studies have defined TGF-β as a critical regulator of thymic T cell development as well as a crucial player in peripheral T cell homeostasis, tolerance to self antigens, and T cell differentiation during the immune response (Li and Flavell 2008). Certain reports indicated that TGF-β has been implicated in the development of thymus-derived naturally occurring Treg cells (nTreg) (Liu et al. 2008). In addition, TGF-β promoted the differentiation of induced Treg (iTreg) cells from naïve T cells in peripheral tissues, which is enhanced by retinoic acid and IL-2 (Fig. 12.3) (Benson et al. 2007; Coombes et al. 2007; Davidson et al. 2007; Mucida et al. 2007;

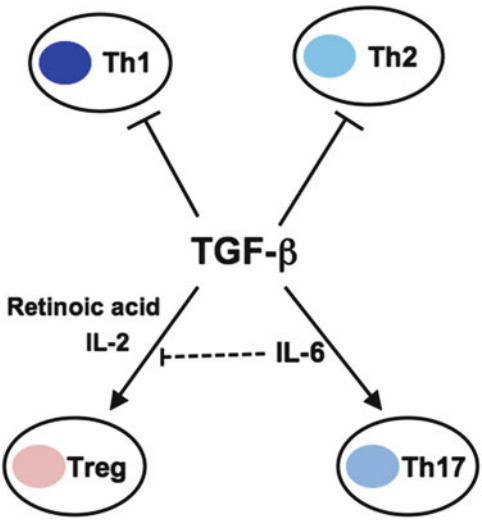

Fig. 12.3 A role of TGF-β in T cell differentiation and tolerance. Active TGF-β directly inhibits the differentiation of Th1 and Th2 cells. TGF-β promotes the generation of the Th17 cells from naïve T cells in the presence of IL-6 and the differentiation of naïve T cells into iTreg cells is simultaneously inhibited

Zheng et al. 2007). It has been known that TGF-β induces the expression of Foxp3, a master regulator of Treg cells (Chen et al. 2003). Foxp3 expression in Treg cells inhibited secretion of pro-inflammatory cytokines, including IL-2, IFN-γ, IL-4, and IL-17. Foxp3 expression also enhanced expression of anti-inflammatory cytokines, IL-10 and TGF-β, and increased an inhibitor for co-stimulation, CTLA4 (Bettelli et al. 2005; Fontenot et al. 2003; Zhou et al. 2008), eventually controlling and dampening inflammation as well as promoting tolerance. In particular, the finding that TGF-β in breast milk induces tolerance through $CD4^+$ Treg cells strongly supports the importance of TGF-β-Treg axis in the immune system (Verhasselt et al. 2008). That is, airborne antigens were efficiently transferred from the mother to the neonate through breast milk and breastfeeding-induced tolerance during lactation was mediated by $CD4^+$ Treg cells depending on the presence of TGF-β (Verhasselt et al. 2008). In addition, it has been reported that TGF-β administrated through the oral route retains sufficient biological activity in the intestinal mucosa and enhances the induction of oral tolerance (Ando et al. 2007). These studies collectively emphasize the crucial role that TGF-β plays to control the immune responses, such as tolerance induction.

While the differentiation of iTreg cells by TGF-β is inhibited in the presence of pro-inflammatory cytokine IL-6, TGF-β promotes the generation of the Th17 cells from naïve T cells in the presence of IL-6 (Fig. 12.3) (Bettelli et al. 2006). When naïve T cells differentiated into Th17 cells upon TGF-β plus IL-6, the differentiation of naïve T cells into iTreg cells was simultaneously inhibited (Fig. 12.3). This differential regulation of naïve T cells by TGF-β implies that the two T cell lineages are closely interconnected showing a mutually exclusive pattern, and has important functions in immune responses. The mechanisms underlying the regulation of Th17 and iTreg cell differentiation remains to be understood.

Alternatively, another study showed that dendritic cells (DCs) are involved in T cell responses regulated by TGF-β. Upon stimulation of naïve T cells by DCs in the presence of TGF-β, antigen-specific Foxp3$^+$ Treg cells were generated (Yamazaki et al. 2006). Treg cells secreted the latent form of TGF-β1 associated with the latency-associated protein (LAP) (Nakamura et al. 2001; Oida et al. 2003), although it is still controversial whether Treg cells are the primary source for TGF-β production. In turn, αvβ8 integrin, which was expressed on DCs, degraded LAP protein through interaction with LAP and released active TGF-β (Taylor 2009). Conditional deletion of integrin αvβ8 on DCs showing autoimmune diseases (Travis et al. 2007) strongly supported the importance of interaction between Treg and DC cells. Active TGF-β finally inhibited the differentiation of Th1 and Th2 cells from naïve T cells and promoted the differentiation to iTreg (Fig. 12.3). Furthermore, certain reports indicate that the mRNA of transmembrane protein called glycoprotein A repetitions predominant (GARP) is selectively induced by TCR stimulation in human Foxp3$^+$ Treg cells and the GARP protein acts as a receptor for latent TGF-β (Wang et al. 2008; Probst-Kepper et al. 2009; Stockis et al. 2009; Wang et al. 2009). These results imply that GARP is a highly specific marker which could be used for the identification of activated Treg cells, and the binding of TGF-β to GARP is an important clue for explaining the activation of Treg cells. In conclusion, a number of studies about the roles of TGF-β in T cells using in vivo or in vitro systems strongly emphasize that TGF-β's immunological function might be more important to understand the pathogenesis of human diseases.

12.3 Conclusion

Numerous studies about TGF-β's biological functions suggest that TGF-β exerts powerful anti-inflammatory activity and is the master controller of immune responses. Therefore, TGF-β-mediated signaling pathway should be critically controlled together with other essential intracellular signaling pathways to maintain homeostatic balance under normal physiological conditions. If this homeostatic balance is disrupted by unexpected stimuli or genetic mutations of TGF-β signaling-related genes, human diseases such as autoimmune and inflammatory diseases may develop. The typical examples are inflammatory bowel diseases such as Crohn's disease. Recently, many researchers are interested in autoimmune diseases and inflammatory diseases exerted by Th17 cells. In particular, Th17 cells and their effector molecules such as IL-17, IL-21, IL-22, and GM-CSF are known to be associated with the pathogenesis of rheumatoid arthritis, systematic lupus erythemaosus, multiple sclerosis, psoriasis, IBD, allergy, and asthma (Miossec et al. 2009; Wilke et al. 2011). Although the TGF-β/Smad signaling pathway is extensively studied in T cell differentiation, Treg cells, and Th17 cells, it has not yet been addressed as to how the inhibitory Smads, Smad6 and Smad7, act on the homeostasis of T cells. Therefore, it will be interesting to study whether inhibitory Smads mediate the role of TGF-β in T cell biology.

Drugs have been and are being developed to inhibit TGF-β activity, including receptor kinase antagonists, neutralizing antibodies, and antisense oligonucleotides. Since inhibitory Smads mediate some of TGF-β's anti-inflammatory activities, they may have a therapeutic potential for treatment of TLR4- or inflammation-related diseases. Therefore, if the detailed mechanisms of TGF-β's anti-inflammation activity are revealed in the future, it will be quite promising for drug development targeting or mimicking TGF-β or its signaling components.

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