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TGFβ in T cell biology and tumor immunity: angel or devil?

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Abstract

The evolutionally conserved transforming growth factor β (TGFβ) affects multiple cell types in the immune system by either stimulating or inhibiting their differentiation and function. Studies using transgenic mice with ablation of TGFβ or its receptor have revealed the biological significance of TGFβ signaling in the control of T cells. However, it is now clear that TGFβ is more than an immunosuppressive cytokine. Disruption of TGFβ signaling pathway also leads to impaired generation of certain T cell populations. Therefore, in the normal physiological state, TGFβ actively maintains T cell homeostasis and regulates T cell function. However, in the tumor microenvironment, TGFβ creates an immunosuppressive milieu that inhibits antitumor immunity. Here, we review recent advances in our understanding of the roles of TGFβ in the regulation of T cells and tumor immunity.

Introduction

TGFβ proteins are a family of pleiotropic cytokines that regulate diverse biological processes, including development of organs and tissues, carcinogenesis and immune responses. TGFβ is synthesized in a latent form with a homodimer of TGFβ that is noncovalently linked with the latency-associated protein (LAP). The activation of latent form TGFβ is promoted by a TGFβ activator via LAP degradation or conformational changes. Active TGFβ binds to TGFβ type 2 receptor (TGFβRII) and induces the assembly of the tetrameric TGFβ receptor complex composed of TGFβRII and TGFβ type 1 receptor (TGFβRI), which activates the kinase activity of TGFβRI. Activated TGFβRI phosphorylates transcription factors, mothers against decapentaplegic homolog (SMAD)2 and SMAD3. Phosphorylated SMAD2 and/or SMAD3 form complexes with the common SMAD (SMAD4) that are translocated into the nucleus where they associate with DNA-binding cofactors to regulate the transcription of target genes [1]. In addition, TGFβ can also activate SMAD-independent pathway, including those mediated by mitogen-activated kinase (MAPK), Rho family proteins, Par6 and PP2A phosphatase to induce different cell type-specific SMAD-independent responses [2].

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In mammals, three members of TGFβ family have been identified: TGFβ1, TGFβ2, and TGFβ3, with TGFβ1 being the major regulator in the immune system. TGFβ is involved in the regulation of development, survival and function of many types of immune cells. However, the role of TGFβ in T cell regulation has attracted the most interest due to the discovery of uncontrolled T cell activation and expansion in TGFβ1-deficeint mice [3, 4]. Given that TGFβ is produced in abundance by many types of tumor cells, it is without surprise that TGFβ facilitates evasion of immune surveillance by regulating T cells and other immune cell types in the tumor microenvironment [5]. In this review, we discuss the current understanding of TGFβ regulation of T cell biology and tumor immunity.

The role of TGFβ in T cell biology

TGFβ was initially defined as a negative regulator of T cells by early studies since addition of TGFβ to T cell culture inhibited T cell proliferation [6]. Consequently, mice that lack TGFβ1 and mice with T cell-specific deletion of either TGFβRI or TGFβRII die early of age from systemic autoimmune disorder caused by hyperactivation and enhanced proliferation of T cells [3, 4, 7–9]. These findings thus suggest TGFβ signaling to T cells is critically associated with the maintenance of T cell tolerance. Intriguingly, recent studies have provided evidence to demonstrate that TGFβ also promotes the differentiation, homeostasis and responses of certain T cell populations (Figure 1). This section focuses on a major role of TGFβ in regulation of T cell differentiation and tolerance. We also address the potential of TGFβ-based therapeutics for the treatment of autoimmune disease.

T cell differentiation

TGFβ has been shown to implicate on the development of T cell precursors into mature T cells in the thymus, as well as differentiation of effector T cells in the periphery. In this section, we focus on a major role of TGFβ in the differentiation of conventional T cells ($CD4^+$ and $CD8^+$), regulatory T (Treg) cells, and non-conventional T cells (NKT, and $CD8\alpha\alpha^+$ intestinal intraepithelial lymphocytes [IELs]).

$CD4^+$ T cells—$CD4^+$ helper T (Th) cells play a major role in establishing and augmenting immune responses against pathogens. This is achieved through their production of cytokines that provide help to other cells in the innate and adaptive immune systems. After activation by engagement of TCR to peptide-MHC complex and co-stimulatory signals, naïve $CD4^+$ T cells undergo proliferation and differentiation into various effector Th subsets, which depends on the nature of antigens and cytokine environment. As TGFβ inhibits the differentiation and function of Th1 and Th2 cells (discussed later), we focus on the stimulatory role of TGFβ in the differentiation of Th17 cells, Treg cells and the recently identified Th9 cells.

Th17 cell differentiation—TGFβ has been shown to be required for the differentiation of Th17 cells from naïve $CD4^+$ T cells, as Th17 cells were profoundly diminished or absent in TGFβ-deficient mice [10]. Moreover, T cells that are deficient in TGFβ receptors, and therefore cannot respond to TGFβ, are impaired in Th17 cell differentiation resulting in mice that are protected from EAE [11]. It was found that TGFβ and IL-6 together induce the differentiation of Th17 cells from naïve $CD4^+$ T cell precursors [10, 12, 13]. In addition to

IL-6, IL-21 together with TGFβ provided an alternative pathway for Th17 cell development in the absence of IL-6 [14].

However, some studies argue the necessity for TGFβ in driving Th17 differentiation under certain circumstances. For example, it was reported that TGFβ indirectly promotes Th17 cell differentiation by inhibiting STAT4 and GATA3 expression, which are required for Th1 and Th2 cell differentiation, respectively. Accordingly, IL-6 alone was sufficient to induce Th17 response in $STAT6^{-/-}.Tbet^{-/-}$ mice [15]. In another study, IL-6 or IL-23 in combination IL-1β was shown to induce Th17 cell differentiation from naïve T cells [16]. These results suggest that TGFβ is dispensable for generating Th17 cells under contain circumstances. Although TGFβ/IL-6 and IL-23/IL-6/IL-1β both induce T cells capable of producing IL-17, the pathogenicity of Th17 cells that arise from these two cytokine environments are strikingly different. Th17 cells generated by stimulation with IL-6/IL-1β/IL-23 efficiently caused severe EAE upon transfer, whereas TGFβ/IL-6-induced Th17 cells had no effect [16]. This was likely due to the high level of IL-10 produced by TGFβ and IL-6-induced Th17 cells [17]. Nevertheless, it is generally believed that TGFβ is critical for the differentiation of Th17 cells at least in rodents. The important questions ahead are the underlying molecular mechanisms downstream of TGFβ signaling that mediate IL-17 gene transcription in T cells.

As TGFβ is a differentiation factor for both Treg (discussed below) and Th17 cells, TGFβ synergizes with other cytokines to regulate Treg and Th17 cell development. It was shown that exposure of naïve CD4+ T cells to TGFβ can result in expression of both Foxp3 and RORγt. However, Foxp3 drives the differentiation of Treg cells by inhibition of RORγt function. In contrast, IL-6, IL-21 and IL-23 release RORγt from Foxp3-mediated inhibition, thus inducing Th17 cell differentiation [18]. It has also been shown that TGFβ regulates the differentiation of Treg and Th17 cells in a concentration-dependent manner. Low concentrations of TGFβ together with IL-6 and IL-23 promoted the expression of IL-23R, thus inducing Th17 cell differentiation. However, at high concentrations, TGFβ suppresses IL-23R and favors Treg cell differentiation [18]. Therefore, the developmental pathways of Th17 and Treg cells are mutually exclusive even though they share a common differentiation factor, TGFβ.

Th9 cell differentiation—Apart from the well-characterized Th1, Th2 and Th17 cells, Th9 cells which preferentially produce IL-9 were recently discovered and added to the family of helper T cells [19, 20]. Th9 cells are involved in host immunity against gastrointestinal parasites [21]. In addition, Th9 cells are involved in the antitumor immune response by stimulating tumor-specific CD8+ cytotoxic T lymphocytes (CTLs) differentiation and function [22, 23]. However, Th9 cells are also capable of causing autoimmunity and allergic inflammation [19, 24].

Long before the discovery of Th9 cells, it was shown that TGFβ combined with IL-4 induced IL-9 production in naïve $CD4^+$ T cells and IL-2 was also essential [25]. Unlike other helper T cell subset, transcription factor(s) specific to Th9 cells have not yet been identified. Given that, Th2 cells are capable of producing IL-9 upon exposure to TGFβ [20],

it remains to be determined if IL-9-producing $CD4^+$ T cells represent a separate lineage of helper T cells.

Regulatory T cells—Treg cells, which constitute 5% to 10% of $CD4^+$ T cells, are generated in the thymus (tTreg) and can also be induced from naïve $CD4^+$ T cells in the periphery (iTreg). Treg cells have been considered as the major mediator of peripheral tolerance ever since Sakaguchi and colleagues discovered that adoptive transfer of $CD4^+CD25^+$ T cells suppressed the development of autoimmunity [26]. Apart from suppressing the autoimmune response, Treg cells also have a pivotal role in limiting anti-tumor immunity (discussed in tumor immunity section of this review) and excessive immune responses to non-self antigens such as commensal bacteria in the gut. This is indicated by eradication of tumors or the occurrence of inflammatory bowel disease, followed by removal of Treg cells.

tTreg cell differentiation—The production of functional Treg cells by the thymus was first demonstrated by Sakaguchi and colleagues. They showed that transfer of $CD4^+CD25^+$-depleted mature thymocytes produced various autoimmune diseases in athymic nude mice and that $CD4^+$ splenocytes which contained $CD4^+CD25^+$ T cells completely inhibited the development of autoimmunity [27]. Moreover, manipulation of the thymus such as neonatal thymectomy or adult thymectomy combined with cyclophosphamide induced a variety of autoimmune diseases in genetically susceptible mouse strains [28, 29].

TGFβ signaling was previously thought to be dispensable for the development of tTreg cells. This is because 8–17-day-old TGFβ-deficient or TGFβRII-deficient mice have similar numbers of tTreg cells in the thymus compared to wildtype mice and tTreg cells from TGFβ-deficient mice maintain a normal Foxp3 expression [9, 30]. In contrast to these conclusions, we have shown that T cell-specific deletion of TGFβRI inhibited the development of thymic Treg cells in young mice between 3 to 5 days of age [7], which was confirmed by another study using TGFβRII conditional knockout mice [31]. In reconciliation with earlier studies, we showed that thymic Treg cells rapidly expand in the absence of TGFβ signaling as a result of increased production of, and responsiveness to IL-2 in the thymus, since thymic Treg cells were completely absent in mice deficient in both TGFβRI and IL-2. Furthermore, we recently validated a critical function of TGFβ in the induction of Foxp3 gene transcription in the thymic Treg cell precursors by utilizing multiple physiological experimental approaches in mice *in vivo*. We have revealed that thymic apoptosis drives tTreg development and demonstrated a previously unrecognized apoptosis-TGFβ-Foxp3 axis in the tTreg generation in the thymus. [32]. One study has suggested that TGFβ signaling is crucial for the survival of tTreg cells instead, rather than for their lineage commitment. However, increased tTreg cell death was not observed in neonate or adult thymi of *Tgfbr2*$^{f/f}$.Foxp3-Cre mice, in which TGFβ signaling was abrogated after Foxp3 gene expression was switched on, and their tTreg cell levels were comparable to their wildtype counterparts [32]. Collectively, these data provide a compelling evidence for the vital role of TGFβ signaling in the generation of tTreg cells.

iTreg cell differentiation—Similar to other lineages of T cells, naïve $CD4^+$ T cells can also differentiate into Treg cells in the periphery as shown in several experimental settings.

For example, continuous delivery of peptide using osmotic pump or targeting of antigen to DCs by means of the DEC-205 endocytosis receptor have both been shown to successfully transform naïve T cells into Treg cells in the peripheral lymphoid organs [33, 34]. These periphery-induced Treg cells or iTreg cells display the tTreg cell phenotype (expressing tTreg cell-associated cell surface molecules and transcriptional signature) and possess suppressive activity.

TGFβ plays a pivotal role in the generation and expansion of Treg cells in the periphery. We discovered that TGFβ induced Foxp3 gene transcription in the context of TCR stimulation from peripheral naïve CD4+ T cells [35]. Consistently, transient expression of TGFβ specifically in the pancreatic islets increased the number of Treg cells in the islets and protected against diabetes [36]. *In vivo* induction of Treg cell generation by low dose antigen delivery is also dependent on TGFb [33, 34]. In our most recent studies, we have successfully generated antigen-specific Treg cells in mice with established autoimmunity, which could potently suppress the autoimmune diseases [37]. Mechanistically, binding of SMAD3 and NFAT to the Foxp3 enhancer and/or Basic HLH protein E2A binding at the Foxp3 promoter are required to switch on Foxp3 expression [35, 38, 39]. The induction of Foxp3 was further proven to be mediated by TGFβ-SMAD singling pathway as T cells that lack CNS1 (contains a SMAD-NFAT response element) or SMAD3 binding sites showed reduced iTreg generation [40, 41].

In addition, retinoic acid produced by $CD103^+$ DCs in the gut-associated lymphoid tissue facilitates the generation of iTreg cells in the presence of TGFβ [42–44]. Retinoic acid is suggested to enhance TGFβ-induced Foxp3 expression in naïve $CD4^+$ T cells by suppressing $CD44^{hi}$ effector/memory T cells, which secrete IL-4, IL-21 and IFNγ to restrain iTreg cell generation [45], although it remains in debate [46]. Thus, it is possible that the gut preferentially promotes the generation of iTreg cells through the release of TGFβ and retinoic acid so that iTreg cells can dampen any excessive immune response in the microbe-rich environment.

Although TGFβ signaling is crucial for the development of both tTreg and iTreg cells, it may not be absolutely required for Treg cell function. This was demonstrated in a recent report that TGFβRII-deficient Treg cells prevented the development of diabetes when cotransferred with diabetogenic BDC T cells into lymphopenic Scid/NOD mice [47]. However, we have shown that Treg cells that lack TGFβ signaling (isolated from *Tgfbr1*$^{f/f}$.Foxp3-Cre mice) suppress the development of EAE and asthma but fail to confer their suppressive function in the gut (Konkel, unpublished data). Our results suggest that the requirement of TGFβ signaling in Treg cells function is contingent on the site of action. The exact function of TGFβ signaling in Treg suppressive activity still remains to be elucidated.

$CD8^+$ T cell differentiation—The role of TGFβ signaling in $CD8^+$ T cell differentiation in the thymus is still unclear. We showed a decade ago that mice with TGFβ1 null mutation show reduced frequency of CD8+ T cells in the thymus and periphery [48]. Similarly, mice with T cell-specific deletion of TGFβRII (*Tgfbr2*$^{f/f}$.CD4-Cre) showed a reduced number of single-positive $CD8^+$ thymocytes [8]. This is consistent with our finding that TGFβ signaling induced and maintained CD8α expression in T cells [49]. Nevertheless, another

study reported that *Tgfbr2*$^{f/f}$.CD4-Cre mice developed normal frequencies of single-positive CD4 and CD8 thymocytes [9]. The disparity between the two studies may result from the timing of examination and development of systemic inflammation in *Tgfbr2*$^{f/f}$.CD4-Cre mice, which could mask the effect of TGFβ on the development of CD8$^+$ T cells. Consistent with this notion, a recent study showed that in the absence of autoimmunity, female *Tgfbr2*$^{f/f}$.CD4-Cre.HY mice had significantly less CD8$^+$ T cells compared to HY mice with intact TGFβ signaling [50]. CD8$^+$ T cells from *Tgfbr2*$^{f/f}$.CD4-Cre.HY mice expressed much lower level of IL-7Rα compared to their wildtype counterparts [50]. As IL-7 signaling is critically required for the differentiation of thymic CD8$^+$ T cells [51], TGFβ signaling is likely to induce CD8$^+$ T cell differentiation through the regulation of IL-7Rα expression in thymocytes. Taken together, these results confirm the requirement of TGFβ signaling in CD8$^+$ T cell differentiation. Interestingly, we showed that TGFβ signaling was capable of switching on CD8α expression in mature CD4$^+$ T cells by inhibition of Th-POK expression. This is consistent with another study demonstrating that Th-POK actively maintains CD4$^+$ T cell phenotype by repressing genes of the CD8 lineage [52].

Non-conventional T cell differentiation—Apart from CD4$^+$ and CD8$^+$ T cells, TGFβ signaling has also been shown to be essential in the development of non-conventional T cells including NKT cells and TCRαβ$^+$CD8αα$^+$ IELs. Natural killer T (NKT) cells develop from CD4$^+$CD8$^+$ thymocytes. In contrast to conventional T cells, NKT cells do not recognize antigens presented by MHC molecules. NKT cells express a semi-invariant TCR that recognize lipid antigens presented by the MHC class I-related CD1d molecule. A significant decrease of NKT cell precursors was observed in the thymus of mice with T cell-specific deletion of TGFβRII (*Tgfbr2*$^{f/f}$.CD4-Cre) [8, 9]. Although the mechanisms that regulate NKT cell differentiation are not well understood, it has been demonstrated that TGFβ signaling is involved in multiple stages of invariant NKT (iNKT) cell (a subset of NKT cells) development through both SMAD-dependent and independent pathways [53]. Furthermore, TGFβ signaling is specifically required for the survival and function of IL-17-producing, RORγt + iNKT cells [54].

TGFβ signaling also controls the development of TCRαβ$^+$CD8αα$^+$ IELs. Mice with TGFβ1 deficiency or T cell-specific deletion of TGFβRI showed a reduced population of TCRαβ$^+$CD8αα$^+$ IELs due to an impaired development of the TCRαβ$^+$CD8αα$^+$ IEL thymic precursors. The reduction of TCRαβ$^+$CD8αα$^+$ IEL thymic precursors resulted from enhanced expression of the proapoptotic molecule Bim. In contrast, mice with T cell-specific overexpression of TGFβ1 had an increased population of TCRαβ$^+$CD8αα$^+$ IELs and TGFβ was found to induce CD8α expression in TCRαβ$^+$CD8αα$^+$ IEL thymic precursors by repressing Th-POK expression [49].

The role of TGFβ in maintenance of peripheral T cell tolerance

TGFβ signaling to T cells plays an important role in the maintenance of T cell tolerance. This is evidenced by the development of early onset lethal autoimmune diseases caused by hyperactivation of T cells, and overproduction of proinflammatory cytokines in TGFβ1-deficient mice and mice with T cell-specific deletion of TGFβR [3, 7–9]. The pathology of

these mouse models closely resembles that of mice that lack Treg cells [55]. It has been shown that TGFβ maintains T cell tolerance through intrinsic and extrinsic mechanisms.

Intrinsic mechanisms—The importance of TGFβ in controlling T cell tolerance has been demonstrated using TCR transgenic mice that harbor autoreactive T cells. As expected, T cell-specific deletion of TGFβR in NOD mice that carry BDC2.5 TCR transgene led to accelerated autoimmune diabetes with elevated Th1 cells [47]. However, the deletion of TGFβR in both Treg cells and effector $CD4^+$ T cells (Ox40-Cre), but not Treg cells alone (Foxp3-Cre), induced diabetes in BDC2.5-NOD mice. Furthermore, similar to observation in *Tgfbr2*$^{f/f}$.CD4-Cre mice, transferred Treg cells were not able to inhibit diabetes caused by TGFβRII-deficient BDC2.5 T cells. Collectively, these results suggest that direct regulation of T cells by TGFβ is critical for the maintenance of T cell tolerance.

Although TGFβ has been shown to be critically required for T cell tolerance, the molecular mechanisms by which TGFβ regulates T cells are not well understood. Several reports have proposed the following mechanisms that may contribute to TGFβ-mediated regulation of T cells; TGFβ inhibited the proliferation of target T cells by blocking the production of IL-2 in a SMAD3-dependent mechanism [56]. TGFβ also inhibited the expression of T-bet and GATA-3 which are required for the differentiation of Th1 and Th2 cells, respectively [57, 58]. This is consistent with findings that $CD4^+$ T cells deficient in TGFβ signaling differentiated into Th1 and Th2 cells [8, 9, 59]. Apart from inhibiting T cell proliferation and differentiation, TGFβ has also been shown to prevent T cell activation by blocking TCR signaling events, including the activation of the Tec kinase Itk, calcium influx and translocation of NFAT [60]. TGFβ also directs the differentiation of naïve T cells into Treg cells which further enforce peripheral T cell tolerance [35]. A recent report has demonstrated that TGFβ prevented an inflammatory response in the gut by downregulating ThPOK and upregulating Runx3. The transition between CD4 and CD8-lineage transcription factors switched on the expression of CD8α in the colitogenic $CD4^+$ T cells and modulated intestinal inflammatory response [49, 61].

Extrinsic mechanisms—$CD4^+Foxp3^+$ Treg cells play an important role in the maintenance of peripheral T cell tolerance as evidenced by the development of catastrophic autoimmune disease in mice and humans with Foxp3 mutations [62, 63]. Treg cells have been shown to produce inhibitory cytokines such as TGFβ and IL-10, which have potent immunosuppressive effects. It has been demonstrated that Treg cells mediate suppression *in vitro* by delivering membrane-bound TGFβ to responder T cells via a cell contact-dependent manner [64, 65]. Moreover, Treg cells that express membrane TGFβ delay the progress of diabetes by inhibiting the migration of $CD8^+$ T cells into pancreatic islets [66]. It has also been suggested that Treg cells require membrane-bound TGFβ to convert naïve T cells to Treg cells via the mechanism of infectious tolerance [67]. These findings are consistent with studies *in vivo*. T cells deficient in TGFβRII are resistant to Treg cell suppression and cause colitis or diabetes, suggesting that TGFβ responsiveness in self-reactive T cells is crucial for Treg cell suppression [47, 66, 68]. Importantly, Treg cells from TGFβ-deficient mice retain the ability to protect against colitis but the protection is abrogated by anti-TGFβ antibody [68]. This result therefore raises the possibility that TGFβ is required for Treg cell-mediated

suppression *in vivo* but that TGFβ may be derived from non-Treg cells. One study demonstrated that TGFβ$^{-/-}$ Treg cells were as suppressive as wildtype Treg cells when these cells were cultured with wildtype antigen presenting cells (APCs) [30]. However, a significantly reduced suppression by TGF-β$^{-/-}$ Treg cells was observed when the cells were stimulated with TGF-β$^{-/-}$ APCs, indicating that TGFβ produced by APCs may aid Treg suppressive activity. However, other studies have revealed that TGFβ produced by Treg cells is required for their suppressive function *in vivo*, as Treg cells from mice with T cell-specific deletion of TGFβ1 failed to inhibit colitis [69]. Furthermore, anti-TGFβ antibody blocked the therapeutic effect of transferred Treg cells on colitis or airway allergic responses [70, 71]. The discrepancies between these *in vivo* studies remain unresolved. More work is needed to define the role of secreted and membrane-bound TGFβ, as well as the source of secreted TGFβ in Treg suppression.

TGFβ-based therapeutics for the treatment of autoimmune disease

Given the role of TGFβ in regulating T cell tolerance, TGFβ represents a potential therapeutic target in the treatment of autoimmunity. Early studies have demonstrated that *in vivo* administration of TGFβ decreased the incidence and severity of EAE, even when TGFβ was administered after disease onset [72, 73]. However, systemic treatment of TGFβ may not be practical in clinical practice due to the pleiotropic roles of TGFβ in many cellular pathways, including differentiation, proliferation, function and homeostasis. Importantly, administration of TGFβ in patients may lead to worsening of existing autoimmune inflammation due to increased differentiation of pathogenic Th17 cells in the presence of proinflammatory cytokines such as IL-6.

An alternative method to apply TGFβ in the treatment of autoimmune disease would be through adoptive transfer of Treg cells, as TGFβ produced by Treg cells is critical for controlling T cell tolerance with the added advantage of antigen specificity while avoiding overall immunosuppression. Indeed, adoptive transfer of Treg cells has resulted in successful prevention of graft-versus-host disease in human clinical trials [74, 75]. However, the effectiveness of Treg cells to treat established autoimmune diseases is less satisfactory. Mice with ongoing disease were not completely cured after treatment with Treg cells due to the persistence of pathogenic activated/memory T cells that are more resistant to suppression by Treg cells [76–78]. This is likely to be caused by intrinsic properties of the effector T cells, such as production of high levels of pro-inflammatory cytokines. Indeed, previous studies have shown that while Treg cells are effective in suppressing naive autoreactive T cells, they fail to control pathogenic effector T cells that secrete IL-17, IFNγ, IL-6 and TNFα [79–81]. Some autoimmune diseases like type 1 diabetes can be predicted or diagnosed at an early pre-clinical stage by the presence of islet autoantibodies in individuals with high-risk genetic markers. However, many others such as autoimmune gastritis, autoimmune hepatitis, and multiple sclerosis are difficult to diagnose since they may be asymptomatic at the early stages or share symptoms in common with diseases without an autoimmune basis. As a result, an early diagnosis for autoimmune diseases is not always possible and treatment that can reprogram dysregualted immune response in advanced disease will be critically required. Furthermore, the generation and expansion of Treg cells

(especially autoantigen-specific Treg cells) in adequate numbers for adoptive transfer may be difficult in a human setting.

To this end, we have recently developed a new therapeutic approach that induces the generation of autoantigen-specific Treg cells in *vivo* [37]. We initially induced apoptosis of immune cells in mice with established autoimmune diseases by systemic sub-lethal irradiation, or depleted B and $CD8^+$ T cells with specific antibodies. This removes a substantial proportion of pathogenic cells before reestablishment of immune tolerance. Also, apoptotic cells induced professional phagocytes to produce TGFβ that contributed to an immunosuppressive milieu, as shown previously [82]. Auto-antigenic peptides were then administered into the treated mice to promote the generation of autoantigen-specific Treg cells. We have demonstrated that this therapeutic approach successfully ameliorates disease in EAE and NOD models.

TGFβ in tumor immunity

TGFβ is a powerful cytokine whose overall actions greatly depend on the physiological setting. While the requirement for TGFβ in maintaining self-tolerance is undisputable, it is also a critical component in the aetiology of cancer. As a testament to its pleiotropic effects on immune and non-immune cells alike, TGFβ can function both as a tumor suppressor and a tumor promoter. TGFβ initially functions as a tumor suppressor in the early stages of carcinogenesis by inhibiting cancer cell proliferation. However, the tumor later becomes refractory to the growth-inhibitory effects of TGFβ due to the accumulation of mutations that inactivate TGFβ receptors or downstream signaling and it ultimately fosters an environment conducive for tumor progression [83, 84].

The high level of TGFβ in the tumor microenvironment (TME) also plays another major role: evasion of immune surveillance [85]. Local immunosuppression in the TME appears to underlie the failure of a vast array of cancer therapies, and is highly specific in nature, since tumor-bearing animals respond normally to challenge with non-tumor antigens [86]. TGFβ in the TME can suppress or alter activation, maturation and differentiation of both innate and adaptive immune cells, including NK cells, DCs, macrophages, neutrophils and $CD4^+$ and $CD8^+$ T cells [87, 88]. Additionally, TGFβ-induced Treg cells in the TME further contribute to the tolerizing environment. This section will outline the effects of TGFβ on various populations of immune cells in the TME, as well as outline efforts to block TGFβ signaling in combination with immunotherapy.

Effect of TME TGFβ on innate immunity

Natural killer cells—Natural killer (NK) cells represent a critical component of the innate immune system, and function by inducing cytolysis in infected cells through granzyme or perforin release, and boosting the maturation and activation of DCs, macrophages and T cells through IFN-γ and TNFα secretion [89]. TGFβ has been shown to inhibit expression of NK cell activating receptors and this downregulation is associated with reduced cytotoxic granule release, IFN-γ secretion and tumor killing, and an overall poor clinical prognosis [90–92]. This can be attributed to a direct effect of TGFβ or might result indirectly from interaction between NK cells and Treg cells which produce this cytokine [93]. The finding

that TGFβ can suppress IFN-γ through SMAD3-dependent signaling provides evidence for the former mechanism [92].

Tumor antigen-specific NK cells have demonstrated effective anti-tumor activity *in vitro* and are able to infiltrate solid tumors *in vivo*, however, their activity is considerably weakened in the tumor microenvironment [94, 95]. It has been further demonstrated that TGFβ blunts the activation of NK cells in human ovarian cancer by antagonizing IL-15 induced proliferation and gene expression (normally associated with NK cell activation) [96]. Expectedly, TGFβ-blockade restored NK cell activation and effector function in this context. Intriguingly, TGFβ seems to only affect activation, and not survival of NK cells, in contrast to its pro-apoptotic effects on T cells [97]. Thus the TGFβ-rich TME maintains an inactive but viable population of NK cells, whose activity can be restored by TGFβ-blockade. Indeed, antagonism of TGFβ signaling has been shown to result in restoration of normal levels of NKG2D and concomitant anti-tumor functionality in NK cells [98]. Increased secretion of IFN-γ in these NK cells may also aid in anti-tumor immunity, as antagonism of TGFβ signaling has been shown to promote the accumulation of NK cells that are able to secrete high levels of IFN-γ [99]. These findings suggest that NK cells may serve as an untapped subset of cells that can be used to restore anti-tumor immunity.

DCs—Dendritic cells (DCs) are unique in their ability to induce primary immune responses in the establishment of immunological memory and are considered the most effective antigen-presenting cells [100]. A number of reports have demonstrated that tumor-infiltrating DCs are defective in their ability to activate anti-tumor T cell responses in a variety of human cancers [101–103]. This may be due in part to high TGFβ levels in the tumor microenvironment. Tumor-derived TGFβ has been shown to immobilize DCs and prevent migration to tumor-draining lymph nodes in mouse and human skin cancers [104, 105] and may also directly induce DC apoptosis in tumor-draining lymph nodes [106]. Importantly, DCs can present antigen in an immunogenic or tolerogenic manner, depending on the local micro milieu and cytokine environment, and thus play a key role in determining the overall response to tumors [107, 108]. In TGFβ-rich environments like the TME, DCs take up tumor cells, become tolerogenic TGFβ-secreting cells and promote the induction of tumor-specific Treg cells in both mice and humans [109–112] that in turn act as potent inhibitors of anti-tumor T cell responses [113, 114].

DCs serve as an attractive target in cancer immunotherapy due to their potency as APCs. Indeed, since Treg cells are one of the major obstacles to successful antitumor immunity, the ability of DCs to promote Treg development in the TME presents the possibility of indirectly downregulating these immunosuppressive cells by targeting DCs. So far, a number of protocols for the generation of clinical-grade DCs for use in cancer vaccines have been generated [115, 116]. However, only modest clinical efficacy has been observed, likely due to high TGFβ levels in the TME, which can significantly affect the responsiveness of T cells to DC priming [117]. Thus simultaneous neutralization of TGFβ during administration of DC-based vaccines may further enhance the ability of DC-primed T cells in tumor eradication. However, since TGFβ can inhibit tumor proliferation in certain contexts, systemic inhibition of TGFβ may lead to accelerated tumor growth. The challenge now is to

design strategies for localized neutralization of TGFβ in the TME, so as to reverse immunosuppression without blocking the inhibitory effect of TGFβ on tumorigenesis.

Macrophages and myeloid-derived suppressor cells—Macrophages exhibit a remarkable degree of plasticity and adopt either pro- or anti-inflammatory phenotypes in response to environmental stimuli [118]. Classically activated macrophages (M1 macrophages) mediate host defense to invading pathogens and elicit anti-tumor immunity. Alternatively activated macrophages (M2 macrophages) have anti-inflammatory functions and regulate wound healing. Macrophages constitute a major component of immune cell infiltrate in the TME, and can constitute up to 50% of tumor mass. In the vast majority of human cancers, high frequencies of tumor-associated macrophages (TAMs) correlate with poor prognosis [119, 120]. These TAMs are generally believed to have an M2-like phenotype [118, 121], although it is not unanimously accepted. In addition, the absence of M1-orienting signals such as IFN-γ as well as pro-M2 stimuli such as IL-10 and TGFβ in the tumor TME may skew differentiation of macrophages toward the M2 phenotype [122]. TAMs are best characterized by their ability to suppress anti-tumor immunity and are associated with increased expression of arginase 1 and indoleamine 2,3-dioxygenase (IDO) that inhibit T cell proliferation and survival, and themselves contribute to high IL-10 and TGFβ levels [123]. Global gene profiling of TAMs highlighted upregulation of a number of genes. In particular, migration-stimulating factor (MSF) is induced in TAMS by CSF-1, IL-4 and TGFβ [124]. TAM-derived MSF strongly stimulated tumor cell migration, contributing to the increased motility of neoplastic cells. Thus the TGFβ-rich TME may favor M2-polarization of macrophages, which in turn reinforces the metastatic properties of the tumor. Another mechanism by which TGFβ can circumvent anti-tumor responses is by negatively regulating Toll-like receptor (TLR) signaling in macrophages. Recognition of microbial signatures such as LPS, DNA and lipopeptides by TLRs activates MyD88 and TRIF signaling pathways in macrophages, which result in secretion of several cytokines involved in the anti-tumor responses, such as TNFα, IL-12 and IFN-γ [125]. TGFβ can induce expression of the interleukin receptor associated kinase (IRAK-M), a key negative regulator of TLR signaling [126]. While IRAK-M is critical for preventing excessive inflammatory responses [127], its presence is detrimental to in the context of cancer due to its potential to induce evasion of host immune surveillance [128]. Indeed, IRAK-M gene expression was found to correlate with poor survival in lung cancer sufferers [126]. Thus, taken together, TGFβ in the TME may act first to polarize macrophages towards an immunosuppressive subtype, and in continuous manner to reinforce this phenotype through crosstalk with TLRs.

It is easy to dismiss the fact that macrophages are essentially “eaters”, and their anticancer potential has until more recently been underestimated. Tumor cells subvert engulfment by transmission of “don’t eat me” signals to macrophages [129]. Indeed, the voracious phagocytic properties of macrophages have been harnessed in the treatment of pancreatic cancer in humans [130] and in the engulfment of various mouse tumors [131]. However, phagocytosis of apoptotic tumor cells by TAMs may also outcompete tumor antigen uptake by DCs, indirectly limiting anti-cancer T cell responses [132]; TGFβ has been shown to enhance these phagocytic properties of TAMs [133]. Fortunately, the differentiation of macrophages is possibly reversible depending on the microenvironment [134–136],

suggesting that TAMs have the potential to be deconverted into an anti-tumor M1-like phenotype with a change in cytokine environment. Thus one can envision a macrophage-based anti-cancer therapy in which neutralization of TGFβ can 1) prevent tumor immune evasion due to reduced competition for tumor antigen, and 2) reprogram TAMs into an anti-tumoral M1-phenotype to activate adaptive immune responses to the tumor. Simultaneous administration of therapies to enhance “eat me” or downregulate “don’t eat me” signals in cancer cells would then unleash the phagocytic properties of these cells, which, given their large numbers in the TME, are ideally placed to “eat cancer”.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of activated immature myeloid cells that are characterized by a mixture of granulocytic and monocytic cells but lack the expression of cell-surface markers associated with fully differentiated monocytes, macrophages or DCs [137]. MDSCs have been found to undergo expansion and accumulate in the tumor host [138, 139]. As its name suggests, this population of cells is highly immunosuppressive [138, 140, 141], which may contribute greatly to the tumor progression. MDSC represent an important component of the immune-suppressive network responsible for defective anti-cancer T cell responses and also contribute to tumor progression via regulation of angiogenesis and tumor cell motility. These cells are found in increased numbers in the TME, and the peripheral blood, liver, and tumor-draining lymph nodes of the cancer-bearing host, with their frequency correlating with increased stage and metastatic disease [142, 143]. It is thought that MDSCs are recruited and undergo expansion in response to tumor-secreted cytokines like IL-6, GM-CSF and IL-1β, PGE2, VEGF, IDO, IL-10, SCF and importantly TGFβ [144, 145]. At the TME, MDSCs mediate immunosuppression through involve arginase 1 (ARG-1)-mediated depletion of L-arginine, inducible nitric oxide synthase (iNOS) and NADPH oxidase (NOX2)-mediated production of reactive nitrogen and oxygen species, VEGF overexpression, cysteine depletion and secretion of TGFβ [146–150]. Thus MDSCs can both respond to and secrete TGFβ. In addition to directly limiting T cell responses, emerging evidence supports a role for MDSCs in the local expansion and functional maturation of $CD4^+CD25^+Foxp3^+$ Treg cells in the TME, through TGFβ-dependent and independent pathways [109, 112, 151], suggesting that MDSCs can further reinforce the immunosuppressive environment at the tumor site by maintaining Treg cells.

MDSC depletion in the TME has shown promise in mouse models of breast and lung cancer by greatly enhancing $CD8^+$ T cell responses and decreasing Treg cell infiltration [152, 153]. Thus, a major mechanism through which TGFβ-blockade might work is through reduced recruitment and maintenance of this mixture of immunosuppressive cells at the TME.

Neutrophils—Neutrophils are the predominant leukocyte subset in human blood and have a well-established role in first line defense against microbial pathogens. However, due to their short life span and fully differentiated phenotype, neutrophils were thought to have negligible impact on cancer immunology. Neutrophils have more recently emerged as new tumor-infiltrating myeloid cells; named tumor-associated neutrophils (TANs). High TAN infiltration has been associated with poor clinical outcome in several human cancers, such as renal cell and hepatocellular carcinomas, colorectal cancer, and head and neck cancer [154]. Indeed neutrophils have been found to regulate key mechanisms of tumor progression such

as angiogenesis, invasion and metastasis. For example, neutrophils can have strong pro-angiogenic activities via release of matrix metalloprotease (MMP9) and vascular endothelial growth factor (VEGF) [155]. Neutrophils can also promote tumor motility through the release of proinflammatory cytokines that enhance the invasive and migratory potential of tumor cells [156–159]. However, TANs can also be associated with better prognosis in other cancers, such as gastric carcinomas [160]. TANs can exert an anti-tumoral effect through a direct cytotoxic activity against tumor cells and can release a range of mediators (cytokine, chemokines and growth factors) to recruit and activate cells of the adaptive immune system. It is thought that the dichotomous roles of TANs in tumor suppression and promotion can be resolved by careful characterization of TAN subtypes. Analogous to macrophages, TANs can take on a tumor-inhibiting N1 phenotype or a tumor-promoting N2 phenotype [161]. The polarization process appears to be dependent on the microenvironment with TGFβ playing a key role in this respect [162]. While earlier studies initially showed that TGFβ acts directly as a potent chemotactic factor for neutrophils [163], and could also influence neutrophil migration indirectly by regulating the expression of adhesion molecules in the endothelium [164], more recent studies have revealed that TGFβ can also influence the polarization of TANs. In particular, Albelda and colleagues showed that TGFβ drives resident TANs to become tumor-promoting N2 neutrophils associated with an immunostimulatory profile ($TNF\alpha^{high}$, $CCL3^{high}$, $ICAM^{high}$, $arginase^{low}$); in contrast, TGFβ-blockade promoted acquisition of the anti-tumor N1 phenotype [161]. Depletion of this N2 subpopulation in tumor-bearing mice was sufficient to inhibit tumor growth, highlighting the impressive immunosuppressive potential of N2 TANs [161, 165, 166]. Acquisition of the anti-tumor N1 phenotype has also been shown to promote cell death and inhibits tumor growth [161, 167, 168]. In addition, it was shown that removal of N2 TANs or N1 TANs increased or decreased the activation status of intratumoral $CD8^+$ T cells respectively, in further support of the different immunosuppressive or stimulatory functions of TAN subtypes [161].

The use of monoclonal antibodies in cancer therapy has increased dramatically in the past decade [169], of which a central mechanism is the recognition of IgG Fc domains by Fcγ receptors on NK cell, monocytes and neutrophils, to elicit tumor cell killing [170]. Neutrophils offer advantages for use in tumor therapy due to their abundance, which takes away the need for ex vivo expansion. Simultaneous localized administration of TGFβ antagonists may polarize resident TANs into an anti-tumor N1 phenotype, with concomitant increase in $CD8^+$ T cell activation at tumor sites, to reinforce tumor eradication.

Effects of TME TGFβ on adaptive immunity

T cells—$CD8^+$ CTLs are a critical component of anti-tumor immunity due to their ability to carry out cytolytic killing of tumor cells in a tumor-antigen specific manner [171]. Several studies have shown a direct correlation between the ratio of cytotoxic $CD8^+$ T cells to Treg cells and cancer survival [172–175]. TGFβ in the TME has been shown to reduce antitumor $CD8^+$ T cell response by inhibiting the expression of cytotoxic genes, including perforin, granzyme A, granzyme B, Fas ligand and IFNγ. Neutralization of TGFβ restored $CD8^+$ T cell cytotoxicity and led to tumor clearance [176]. In consistence with this study, $CD8^+$ T cells with impaired TGFβ signaling elicited a strong antitumor immune response and

inhibited tumor development. The protective effect was associated with enhanced tumor infiltration and increased proliferation and activity of tumor-infiltrated CTLs [59, 177, 178]. Interestingly, TGFβ can also influence the anti-tumor effect of CTLs by upregulating IL-17 production, although the effect of IL-17 on tumor growth versus immune surveillance remains controversial [179, 180].

$CD4^+$ T cells have been largely overlooked in their involvement in cancer progression, due to the fact that most tumor cells express MHC class I but not class II. Thus, while $CD8^+$ T cells can induce direct tumor cell killing by recognition of tumor peptides presented in an appropriate fashion on MHC class I complexes on tumor cells, the action of $CD4^+$ T cells is unnoticeable in this respect. However, $CD4^+$ T cells are central to adaptive immunity, and a growing number of studies have emphasized these cells in the induction, maintenance and regulation of antitumor immune responses. Tumor-reactive $CD4^+$ T cells have shown efficacy in tumor eradication or slowed tumor progression in a number of mouse cancers [181–185], even in cases where tumors were resistant to $CD8^+$ T cell-mediated rejection [186]. Moreover, studies in TCR-transgenic mice have supported a role for anti-tumor $CD4^+$ T cells in the activation of memory CTLs in vivo [187]. However, differential effects of $CD4^+$ T cells have been documented in other tumor models [188]; and are thought to be attributed to heterogeneity within the $CD4^+$ T cell population.

Th1 cells are thought to play an important role in anti-tumor-immunity, through the release of IFNγ, TNFα and cytolytic granules. In addition, they are thought to aid in the expansion of tumor-antigen specific CTL populations, through CD40/CD40L interaction and release of IL-2 [189]. Indeed, analysis of immune infiltrates in cancer patients has revealed that anti-tumor immunity is typically polarized towards Th1 responses [190], with increased numbers correlating with improved prognosis [191]. In contrast, Th2 cells are thought to impair tumor-specific responses by secreting cytokines that induce T cell-anergy and inhibit T-cell mediated cytotoxicity. Accordingly, increased numbers of Th2 mostly correlate with tumor progression [191]. In addition, tumor-derived TGFβ was shown to inhibit Th1 responses by skewing polarization of infiltrating T cell towards the Th2 cell phenotype, resulting in a less efficient anti-tumor response [192]. However, more recent studies have suggested that both Th1 and Th2 cells can support CTL responses against cancer cells, although Th1 cells seemed more effective by promoting activation of antigen presenting cells [193, 194].

Although the role of Th17 cells in a number of autoimmune diseases is well established, the activity of this T cell subset in cancer is controversial [12]. Higher numbers of Th17 cells have been detected in various human cancers, such as ovarian, pancreatic, renal cell and gastric cancers [195, 196]. In some cases, a direct correlation between Th17 frequencies in the TME and cancer stage has been documented [197]. Other studies suggest that Th17 cells may instead have anti-cancer effects, as cancer survivors and patients with early-stage cancer have high Th17 levels [198].

Treg cells are generally thought to antagonize protective immunity in cancer [199, 200]. Treg cells suppress a wide range of anti-tumor responses, including $CD4^+$ T cells, $CD8^+$ T cells, NK cells and natural killer T (NKT) cells. Several studies have documented an accumulation of Treg cells at peripheral sites and TME of tumor patients, which correlates

with increased tumor burden and poor anti-tumor effector response [201]. Importantly, this is often associated with low $CD8^+$ T cell frequencies at these sites, suggesting that tumor-infiltrating Treg cells dampen anti-tumor responses through suppression of CTLs [201].

The composition of $Foxp3^+$ Treg cells in the TME is poorly understood, though a number of studies suggest that tTreg cells are recruited to the tumor site where they undergo expansion. It was shown that specific recruitment of pre-existing human Treg cells was mediated by high levels of the chemokine CCL22, produced by tumor cells and macrophages in the TME [202]. Importantly, tumor-associated Treg cells were shown to undergo substantial proliferation, in response to TGFβ produced by MDSCs at the tumor site [109]. In further support of this model, the TCR repertoires of tumor-infiltrating Treg and conventional T cells were found to be non-overlapping, indicating that tumor-infiltrating Treg cells were likely tTreg cells, as a significant overlap in TCR repertoires would have been observed from de novo differentiation of naïve T cells into iTreg cells [203]. In contrast, other studies suggest that tumor-infiltrating Treg cells are mostly iTreg cells resulting from the conversion of naïve T cells in response to high levels of TGFβ at the TME [204–206]. However, rather than being mutually exclusive, it is likely that both iTreg and nTreg cells contribute to the total Treg pool in the TME [207].

Not all Treg cells express Foxp3; $Foxp3^-$ Treg cells such as IL-10-secreting Tr1 cells can likewise exert immune-suppressive effects [208, 209]. In particular, Tr1 cells are found to be enriched at the TME in a number of cancers and demonstrate a prominent antitumor response *in vitro* [210–212]. Increased Tr1 frequencies, with concomitant decreases in $FoxP3^+$ Treg cells correlated with a better survival rate in a study using *ex vivo* stimulated PBMCs for treatment of ovarian cancer [213]. Consistent with this, Tr1 cells have shown efficacy in tumor eradication in a murine glioma model by augmenting CTL and NK cell responses [214]. Thus, it has been postulated that the ratio of $Foxp3^+$ Treg cells versus $IL\text{-}10^+$ Tr1 cells may affect antitumor responses, although further studies are required to study the role of Tr1 cells in antitumor immunity.

Important considerations in TGFβ-based therapies for cancer

The tumor site represents a unique microenvironment with a variety of cell types, including neoplastic cells, stem-like cells, fibroblasts, endothelial cells and immune cells, all engaged in some level of cross-talk with each other. Although tumor cells are known to secrete TGFβ, immune cells such as effector T cell, Treg cells, APCs and MDSCs may well represent a large source of this cytokine. Identifying the most relevant source of TGFβ remains an important quest, albeit a difficult one given that this may vary with stage and site of the cancer. Moreover, given its pleiotropic effects on a large range of cells, immune and non-immune alike, it is unclear as to which of the TGFβ-mediated effects dominates in the TME. Nonetheless, the effect of TGFβ on immune cell infiltrate at the tumor site seems to be an overall suppressive and anti-proliferative one. Thus, TGFβ-blockade may act to unleash the antitumor effects of key components of the innate and adaptive immune system. For example, simultaneous inhibition of TGFβ-signaling with adoptive T cell therapy has been shown to significantly improve T cell survival and anti-tumor T cell cytotoxicity [215]. Further study is then needed to identify the ideal timing of TGFβ blockade in the host, as

well as optimize delivery of inhibitors in a localized fashion at the TME to avoid deleterious systemic effects. Importantly, TGFβ is instrumental in the differentiation programs of numerous immune cell types, for example, instructing the M1/M2-polarization of macrophages and conversion of naïve T cells into Treg cells, further reinforcing an immune cell environment conducive to tumor progression. Clearly, more studies are needed to clearly delineate the functions of different immune cell subsets in cancer, particularly, the ambiguous role of Th17 cells at the TME. Once this is elucidated, however, localized TGFβ-inhibition would then provide the potential to skew the polarization of different immune cells towards a more immunogenic, anti-tumoral phenotype at the TME, effectively allowing us to harness our own inherent defenses to combat this devastating disease.

Concluding remarks

It has been two decades since Doetschman and colleagues made the seminal observation of catastrophic inflammatory disease in TGFβ1-deficient mice [3]. In the meantime, significant progress has been made in our understanding of immune regulation by TGFβ. It is now clear that TGFβ plays an indispensable role in the immune system, and can act in either a stimulatory or inhibitory manner, depending on the nature and differentiation status of immune cells, as well as the microenvironment and cytokine milieu. However, many fundamental questions remain unaddressed. It is not completely understood as to how TGFβ signaling coordinates Treg, Th17 and Th9 cell differentiation. Furthermore, the mechanisms which dictate whether the TGFβ signal results in T cell tolerance or the differentiation of effector T cells are not yet determined. Although much effort has been directed towards elucidating the role of TGFβ in the context of T cell, TGFβ undeniably plays a major regulatory role for other immune cell types and should not be overlooked in this regard. Understanding the mechanisms by which TGFβ regulates T cells and other components of the immune system will valuable provide insight into the aetiology of autoimmune disease and cancer, knowledge of which is essential for the design of more efficient therapies.

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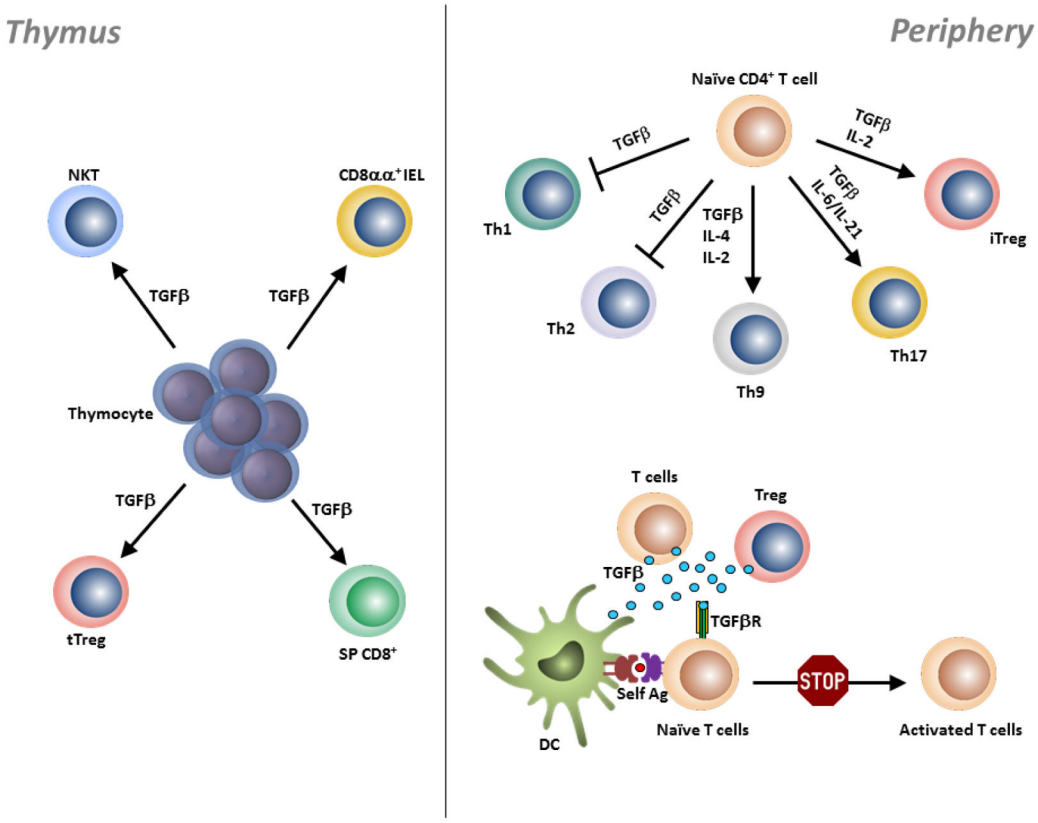

Figure 1. TGFβ regulation of T cells in the thymus and periphery

During T cell development in the thymus, TGFβ supports the differentiation of thymocytes into tTreg cells, CD8 T cells, NKT cells and TCRαβ^{+}CD8αα^{+} IEL precursors. In the periphery, TGFβ inhibits Th1 and Th2 cell differentiation by repressing T-bet and GATA-3 expression, respectively. In other scenarios, TGFβ acts synergistically with other cytokines to promote the differentiation of Th9, Th17 and iTreg cells. DCs, T cells and Treg cells serve as a source of TGFβ, which is critically required for the maintenance of peripheral T cell tolerance by inhibiting activation and proliferation of self-reactive T cells.

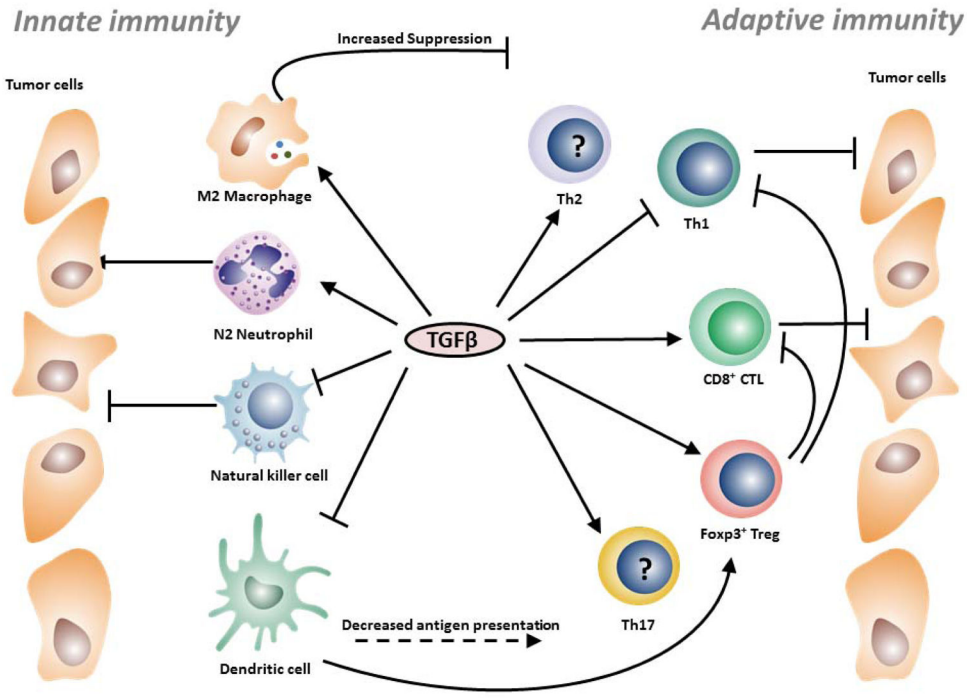

Figure 2. The effect of TGFβ on innate and adaptive immune cells

TGFβ has an overall inhibitory effect on innate immune cells. TGFβ may skew M2-polarization of macrophages, which inhibits T cell proliferation and survival. TGFβ can also convert N1 neutrophils into the less cytotoxic N2 phenotype. The expression of activating receptors in NK cells is inhibited in response to this cytokine, resulting in reduced tumor cell killing. TGFβ in the TME decreases antigen presentation of DCs and provides a tolerogenic environment in which DCs promote tumor-specific Treg cells. Cells of the adaptive immune system display differential responses to TGFβ. Th1 cells are thought to have stronger anti-tumor responses and TGFβ may skew polarization infiltrating T cells at the TME towards the less-efficient Th2 phenotype. Importantly, TGFβ downregulates cytolytic killing of tumor cells by $CD8^+$ CTLs by suppressing their cytotoxic program or blocking TCR signaling. TGFβ promotes differentiation of Th17 and Treg cells, although the role of the former subset in cancer remains controversial. In contrast, Treg cells suppress a wide array of antitumor activities, notably, by inhibiting Th1 and $CD8^+$ CTL responses.