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Brief Reviews

The Journal of Immunology

TGF-β: Guardian of T Cell Function

Soyoung A. Oh and Ming O. Li

A fundamental aspect of the adaptive immune system is the generation and maintenance of a diverse and self-tolerant T cell repertoire. Through its regulation of T cell development, homeostasis, tolerance, and differentiation, the highly evolutionarily conserved cytokine TGF-β critically supports a functional T cell pool. The pleiotropic nature of this regulation is likely due to the elaborate control of TGF-β production and activation in the immune system, and the intricacy of TGF-β signaling pathways. In this review we discuss the current understanding of TGF-β regulation of T cells.
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The highly evolutionarily conserved cytokine TGF-β has three known mammalian family members (TGF-β1, -β2, and -β3) that regulate multiple physiological processes. TGF-β is synthesized in a latent form that must be activated to allow for engagement of a tetrameric receptor complex composed of TGF-βRI and TGF-βRII. The production and activation of TGF-β can be mediated by distinct cellular sources, providing additional complexity to the regulation of this pleiotropic cytokine. Binding of active TGF-β to its receptor complex triggers receptor serine/threonine kinase activity, allowing for the phosphorylation of downstream signaling targets. TGF-β signaling is primarily mediated through the Smad family of transcription factors, but it is also known to engage Smad-independent pathways.

TGF-β1 is the primary isoform expressed in the immune system, and its widespread regulatory activity affects multiple types of immune cells (1). T cells were established as critical targets of TGF-β in its control of immune tolerance by the finding that mice with T cell–specific deletion of *Tgfbr2* phenocopied the lethal inflammatory disorder that develops in *Tgfb1* knockout mice (2–5). Nevertheless, TGF-β is more than an immunosuppressive cytokine. For instance, early studies revealed that TGF-β induces stimulatory or inhibitory effects in human T cells, which is dependent on the T cell differentiation status and the stimulation conditions (6). This context-dependent function of TGF-β allows for its distinct roles in T cell development, homeostasis, tolerance, and differentiation (Fig. 1). In this review we discuss the current understanding of TGF-β regulation of T cells with a focus on recent discoveries.

T cell development

During thymic development, T cell precursors undergo an orchestrated series of changes resulting in the differentiation of distinct mature T cell subsets. TGF-β has been shown to play important roles in the development of conventional, regulatory, and innate-like T cells.

$CD8^+$ T cells. In addition to TCR engagement, signaling via the common γ-chain family cytokine IL-7 is critical for the thymic development of $CD8^+$ T cells (7). Whether TGF-β plays a role in $CD8^+$ T cell lineage commitment was unclear given contradictory reports of reduced and normal thymic $CD8^+$ T cell populations in mice with T cell–specific deletion of *Tgfbr2* during the $CD4^+CD8^+$ thymocyte stage (4, 5). Although TGF-βRII–deficient mice were analyzed before the onset of overt autoimmunity, the confounding effects of systemic inflammation were a concern in both studies. The generation of mice with T cell–specific *Tgfbr2* deletion and an HY transgenic TCR-restricted repertoire allowed for the study of TGF-β regulation of $CD8^+$ T cell development in the absence of autoimmune inflammation. The HY TCR recognizes a male mouse-specific Ag, but it is also positively selected by low-affinity self Ags in female mice. TGF-βRII–deficient female HY transgenic mice exhibited impaired $CD8^+$ T cell development relative to their wild-type counterparts (8). Intriguingly, TGF-β promoted the specification of $CD8^+$ T cell fate largely through its control of thymocyte IL-7Rα expression (and by extension IL-7 signaling) by suppressing the transcriptional repressor Gfi-1, a known *Il7ra* inhibitor in $CD8^+$ T cells (9). These findings reveal a mechanism of $CD8^+$ T cell lineage commitment via the crosstalk of TGF-β and IL-7 cytokine signaling pathways.

Strong agonist ligand–induced selection: regulatory and innate-like T cells. A combination of stringent TCR interactions, costimulation, and cytokine signals controls the development of thymus-derived $CD4^+CD25^+Foxp3^+$ regulatory T cells (tTregs), which are essential for immune tolerance (10). TGF-β signaling was thought to be dispensable for tTreg development based on reports that mice with T cell–specific *Tgfbr2* deletion possessed normal thymocyte $Foxp3^+$ populations (4, 5). However, the

Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065

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Address correspondence and reprint requests to Dr. Ming O. Li, Immunology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065. E-mail address: lim@mskcc.org

Abbreviations used in this article: CNS1, conserved noncoding sequence 1; dLck-Cre, distal Lck-Cre; DNRII, dominant-negative TGF-βRII; EAE, experimental autoimmune encephalomyelitis; GARP, glycoprotein A repetitions predominant; IEL, intraepithelial lymphocyte; iNKT, invariant NKT; LAP, latency-associated protein; pTreg, peripheral regulatory T cell; RIP-mOva, membrane-bound OVA under the control of rat insulin promoter; ROR, retinoic acid–related orphan receptor; Treg, regulatory T cell; tTreg, thymus-derived regulatory T cell.

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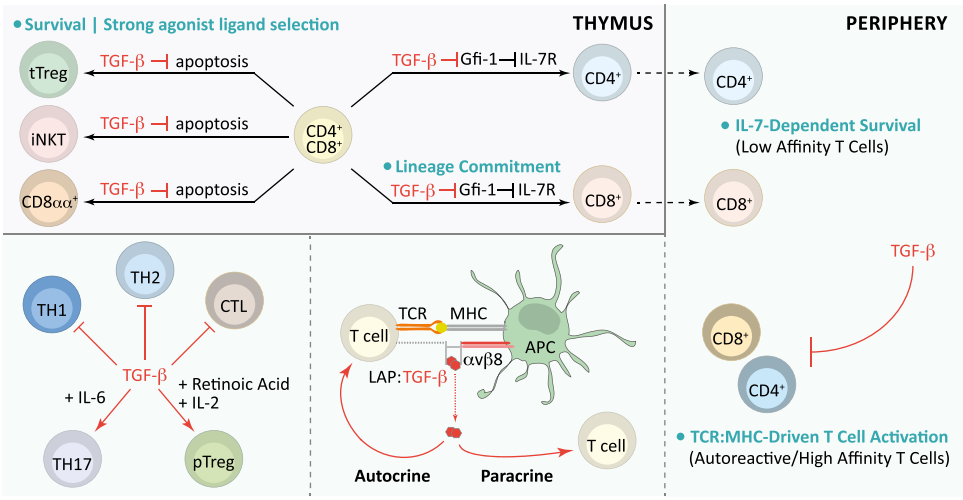

FIGURE 1. TGF-β regulation of T cells. During thymic development, TGF-β–supported survival of tTregs, iNKT cells, and CD8αα$^+$ T cell precursors fosters agonist ligand–induced T cell development. TGF-β also critically regulates thymocyte IL-7R expression by inhibiting the transcriptional repressor Gfi-1, and it promotes conventional CD8$^+$ T cell lineage commitment. In the periphery, TGF-β regulates T cell homeostasis by promoting IL-7–dependent survival of low-affinity T cells (resulting from thymic conditioning of IL-7R expression) and by inhibiting TCR-driven activation of autoreactive/high-affinity T cells. The T cell and APC pairing is essential for in vivo TGF-β function. T cells are a critical source of TGF-β, and APCs engaging T cells by TCR/peptide–MHC interactions are key providers of the $\alpha_v\beta_8$ integrins required for TGF-β activation. Activated TGF-β regulates T cells by autocrine and paracrine signaling. TGF-β inhibits Th1, Th2, and CTL differentiation, but in concert with other factors promotes Th17 or pTreg differentiation.

finding that Foxp3$^+$ thymocyte frequency was dramatically reduced in 3- to 5-d-old mice with T cell–specific *Tgfbr1* deletion indicated that TGF-β does contribute to early tTreg development (11). IL-2–driven expansion of existing Foxp3$^+$ thymocytes resulted in increased tTreg frequencies in older TGF-βRI–deficient mice (similar to observations in TGF-βRII–deficient mice). The discovery of a conserved Smad3 binding sequence in the *Foxp3* gene prompted the hypothesis that TGF-β signaling induces Foxp3 expression in tTregs (12). However, deletion of the enhancer region containing the Smad3 binding site (conserved noncoding sequence 1 [CNS1]) or specific ablation of the Smad binding sequence revealed defects in only peripheral Tregs (pTregs), demonstrating that TGF-β is not required for Foxp3 induction in tTregs (13, 14). Rather, TGF-β has been shown to support tTreg development by antagonizing thymic negative selection. TGF-βRII–deficient thymocytes were found to express high levels of proapoptotic molecules, undergo enhanced negative selection, and contain a reduced frequency of Foxp3$^+$ cells (15). Deletion of the proapoptotic molecule Bim prevented enhanced apoptosis of TGF-βRII–deficient thymocytes and rescued tTreg development.

Invariant NKT (iNKT) cells, which possess innate and adaptive-like properties, recognize lipids presented by the MHC class I–like molecule CD1d. iNKT cells arise from CD4$^+$CD8$^+$ thymocytes and, akin to tTregs, are thought be induced by strong agonist ligand interactions (16). TGF-β signaling was implicated in iNKT cell development based on the observations that thymic and peripheral iNKT cells were lost in mice with T cell–specific deletion of *Tgfbr2* (4, 5, 17). TGF-β was found to promote iNKT cell development in part by inhibiting the apoptosis of immature cells via a transcription intermediary factor 1 γ–dependent, but Smad4-independent, pathway (17). Indeed, constitutive TGF-β signaling led to an increase in iNKT cell frequency; however, the cells were arrested at an immature stage of development, suggesting that although TGF-β signaling promotes this innate-like T cell lineage, it must also be abrogated for proper iNKT cell maturation.

CD8αα$^+$TCRαβ$^+$ intraepithelial lymphocytes (IELs), which are important contributors to intestinal homeostasis, are thought to be generated by both thymic and extrathymic differentiation pathways (18). A recent study reported that enhancing autoreactive thymocyte survival by inhibiting clonal deletion led to increased Runx3-dependent CD8αα$^+$TCRαβ$^+$ IEL development (19), indicating that CD8αα$^+$TCRαβ$^+$ IEL selection is also driven by strong TCR interactions. A role for TGF-β in the development of this innate-like T cell lineage was suggested by TGF-β signaling gain and loss-of-function studies showing increased and decreased frequencies of CD8αα$^+$TCRαβ$^+$ IELs, respectively (20). The loss of CD8αα$^+$ TCRαβ$^+$ IELs in the absence of TGF-β signaling appeared to result in part from a reduction in thymic precursor cells, which were found to express increased amounts of the proapoptotic molecule Bim relative to their wild-type counterparts. Given the role of high-affinity TCR interactions in tTreg, iNKT cell, and CD8αα$^+$ T cell ontogeny, TGF-β–supported survival may be a unifying mechanism that promotes agonist ligand–induced T cell development (21). TGF-β has also been shown to induce CD8α expression on mature CD4$^+$ T cells by regulating the transcription factors ThPOK and Runx3 (20, 22), but whether this pathway promotes Runx3 expression during thymic CD8αα$^+$TCRαβ$^+$ IEL development is unknown.

Naive T cell homeostasis

An effective immune system must establish and maintain a diverse naive T cell pool within the confines of a fairly constant number of peripheral T cells. Critical contributions of TGF-β in the maintenance of T cell homeostasis and repertoire diversity have recently been described.

Low-affinity CD4$^+$ T cells. The severe inflammatory disease that develops in polyclonal mice with T cell–specific TGF-βRII deficiency precludes the precise study of TGF-β control of naive T cell homeostasis (4, 5). In contrast, TGF-βRII–deficient mice with a restricted T cell repertoire in which CD4$^+$ T cells (OVA-specific OT-II) have high affinity for a non-self peptide do

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not develop autoimmunity, allowing for the study of $CD4^+$ T cell homeostasis without the complications of systemic inflammation. The absence of TGF-β signaling did not alter OT-II thymic selection or the naive phenotype of peripheral T cells; however, peripheral T cell frequency was dramatically reduced (4, 8). Impaired homeostasis of TGF-βRII–deficient OT-II T cells was attributed to loss of IL-7Rα expression and the consequent abrogation of IL-7 signaling. Thus, in addition to its role in $CD8^+$ T cell lineage commitment, TGF-β–mediated conditioning of thymocyte IL-7Rα expression critically promotes IL-7–dependent homeostasis of peripheral $CD4^+$ T cells (8). Interestingly, TGF-β was found to be particularly important for the maintenance of low-affinity $CD4^+$ T cells. In the absence of TGF-β, IL-7Rα expression positively correlated with TCR affinity, as TGF-βRII–deficient T cells bearing higher affinity TCRs expressed increased amounts of IL-7Rα (and thus exhibited better homeostatic survival) than did their lower affinity counterparts. Accordingly, a report that TCR diversity is altered only in the periphery, and not in the thymus, of *Tgfb1*$^{-/-}$ mice likely reflects repertoire changes resulting from preferential loss of low-affinity T cells (23). TGF-β maintenance of low-affinity T cells may also be important for a novel regulatory population of "deletor" $CD4^+$ T cells that restricts Ag-specific T cells by competing for endogenous self-peptides, including low-affinity ligands that may mediate positive selection (24).

$CD8^+$ T cells. TGF-β critically regulates thymocyte IL-7Rα expression, and although loss of this conditioning causes impaired CD8-lineage commitment, it likely also has consequences for peripheral $CD8^+$ T cells. Indeed, TGF-βRII–deficient HY $CD8^+$ T cells exhibit dramatic defects in peripheral homeostasis (W. Ouyang and M.O. Li, unpublished observations). Recent studies have also reported altered homeostasis and aberrant activation of TGF-β signaling–defective OT-I T cells (25, 26). Consistent with the finding that TGF-β promotes IL-7Rα expression and IL-7 sensitivity (8), the increased homeostatic proliferation of TGF-β signaling–defective OT-I T cells did not result from enhanced responsiveness to IL-7 (or IL-15). However, although homeostatic proliferation of OT-I T cells expressing the dominant-negative TGF-βRII (DNRII) was freed from classical cytokine requirements, it remained dependent on TCR and MHC class I interactions (25). This requirement for TCR engagement may explain the discrepancies in $CD8^+$ T cell phenotypes from DNRII mice expressing distinct transgenic TCRs. In contrast to the phenotype of OT-I mice, TGF-β signaling–deficient mice with HY or 2C TCR-restricted repertoires did not exhibit an increased frequency of effector or memory-like $CD8^+$ T cells (27, 28). This difference likely results from the increased homeostatic proliferation capacity of OT-I T cells relative to 2C or HY T cells (OT-I > 2C > HY), which is positively correlated with TCR affinity (29). Collectively, these studies suggest that TGF-β plays a dual role in T cell homeostasis by promoting IL-7–driven expansion but inhibiting TCR-driven expansion. By limiting the outgrowth of specific TCRs, TGF-β preserves the naive state and repertoire diversity of the $CD8^+$ T cell pool.

T cell tolerance

The immune system has multiple mechanisms in place to prevent autoimmunity. Central tolerance, whereby overtly self-reactive T cells are deleted in the thymus, is an essential but incomplete part of this process (30). Thus, peripheral tolerance mechanisms have evolved to keep autoreactive T cells in check (31).

The lethal inflammatory disorders that develop in mice with global *Tgfb1* deficiency or T cell–specific deletion of *Tgfbr2* demonstrate the essential role of TGF-β in immune tolerance (2–5). Loss of tolerance in the absence of TGF-β is not solely attributable to defective Treg activity, as the provision of wild-type Tregs (either by adoptive transfer or the generation of mixed bone marrow chimeras) failed to completely rescue the inflammatory disorder in TGF-βRII–deficient mice (4, 5). Indeed, TGF-β has been shown to control peripheral tolerance by both direct and indirect regulation of autoreactive T cells.

Direct regulation of T cells. During thymic development, highly self-reactive T cells undergo negative selection or are diverted to a regulatory lineage. However, these processes are not foolproof, and autoreactive T cells that have evaded central tolerance mechanisms exist in the peripheral T cell pool. Recent studies demonstrate that TGF-β regulation of these "escaped" autoreactive T cells is essential for the maintenance of immune tolerance. In mice with an OT-II T cell–restricted repertoire that also express membrane-bound OVA under the control of rat insulin promoter (RIP-mOva), T cell–specific loss of TGF-β signaling results in enhanced Ag-induced negative selection of OT-II thymocytes (15). Nevertheless, TGF-βRII–deficient OT-II RIP-mOva mice possess peripheral OT-II T cells that eventually induce autoimmune diabetes. In contrast, wild-type OT-II RIP-mOva mice (which have a higher frequency of peripheral OT-II T cells than do their TGF-βRII–deficient counterparts) do not develop diabetes, demonstrating that TGF-β critically regulates diabetogenic OT-II T cells. TGF-β appears to prevent disease by controlling effector T cell differentiation (S.A. Oh and M.O. Li, unpublished observations), which is consistent with recent findings in another transgenic diabetes model (BDC2.5 TCR mice). In BDC2.5 mice, Foxp3-Cre–mediated deletion of *Tgbr2* in Tregs did not induce diabetes, but Ox40-Cre–mediated deletion, which targets both Tregs and activated $CD4^+$ T cells, triggered disease, indicating that direct regulation of diabetogenic T cells by TGF-β is the dominant tolerance mechanism in this model (32). Additionally, in a transfer model of disease, TGF-βRII–deficient Tregs could prevent diabetes, whereas TGF-βRII–deficient effector $CD4^+$ T cells were refractory to suppression (32).

Despite the evidence that TGF-β directly regulates T cells, the exact mechanisms of action remain unclear. One mechanism of TGF-β–mediated tolerance that may be specific to mucosal sites was recently elucidated. In the intestine, TGF-β (with retinoic acid) has been shown to limit inflammation by inducing pathological $CD4^+$ T cells to express CD8α in a manner that requires upregulation of Runx3 and downregulation of ThPOK (22). This redirection of $CD4^+$ T cells to a nonpathogenic phenotype fails to occur in TGF-βRII–deficient T cells.

Although TGF-β is essential for T cell tolerance, it has recently been proposed that loss of TGF-β signaling alone is insufficient to induce autoimmunity. In striking contrast to mice in which *Tgfbr2* deletion occurs during the double-positive thymocyte stage, mice in which *Tgfbr2* undergoes slow deletion (by the distal Lck-Cre [dLck-Cre]) in peripheral

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T cells do not develop autoimmune disease (26). However, adoptively transferred dLck-Cre *Tgfbr2*$^{fl/fl}$ T cells could induce disease in Rag-deficient recipients, suggesting that an additional insult, such as lymphopenia, is required for the development of autoimmunity in the absence of TGF-β signaling. A noteworthy aspect of the dLck-Cre model is that tTregs and iNKT cells, which undergo agonist ligand–induced selection, are reported to express normal amounts of TGF-βRII. This raises the question of whether the dLck-Cre favors deletion in T cells bearing lower affinity TCRs, such that autoreactive CD4$^+$ T cells possessing high-affinity TCRs remain under the control of TGF-β signaling. It would be of interest to compare the expression profiles of TGF-βRII and Nur77GFP, which reflects TCR signal strength (33), to determine the pattern of dLck-Cre activity in T cells.

pTreg controlled tolerance. pTreg induction is driven by a combination of suboptimal TCR signaling and other environmental stimuli, including TGF-β (10). TGF-β promotion of pTreg development can occur in part through the inhibitory effects of TGF-β signaling on TCR activation (34). The key role of TGF-β in inducing Foxp3 expression in pTregs was identified by studies showing that deleting the *Foxp3* CNS1 region (which contains the conserved Smad3 binding sequence) or the Smad binding site alone results in a reduction of pTregs (13, 14). Interestingly, in addition to promoting pTreg development, TGF-β also induces Th17 differentiation (covered later in this review). The disparate effects of TGF-β on pTreg versus Th17 cell fate reflect the context-dependent function of this pleiotropic cytokine. Indeed, other environmental cues, such as IL-2 and retinoic acid for pTregs and IL-6 for Th17 cells, influence the development of a specific lineage. Moreover, certain cell types can also favor pTreg differentiation; CD103$^+$ dendritic cells from gut-associated lymphoid tissues and mesenteric lymph nodes have been shown to preferentially induce pTregs in a TGF-β– and retinoic acid–dependent manner (35–37). On a molecular level, it has been shown that TGF-β can induce the same CD4$^+$ T cells to express both Foxp3 and retinoic acid–related orphan receptor (ROR)γt (the key Th17 transcription factor), but Foxp3-mediated inhibition of RORγt activity, which appears to result in part from physical interactions between the two transcription factors, can favor pTreg development (38).

In contrast to the insights into pTreg differentiation, the unique contributions of this population to immune tolerance have only recently been revealed. The *Foxp3* CNS1 element was found to be highly conserved in placental mammals, and CNS1-deficient female mice bred to allogeneic males exhibited increased fetal resorption relative to wild-type females, indicating that pTregs are important regulators of maternal–fetal tolerance (39). Peripheral Tregs were also shown to regulate tolerance at mucosal sites, as mice lacking CNS1 developed Th2-driven autoimmunity in the gastrointestinal tract and lungs (40).

Effector T cell differentiation

TGF-β can broadly impede T cell activation by inhibiting TCR signaling (34), but it also inhibits specific Th cell subsets by suppressing lineage-defining transcription factors, such as T-bet and GATA-3, which are critical for Th1 and Th2 CD4$^+$ T cell differentiation, respectively (41, 42). Indeed, TGF-β signaling-deficient CD4$^+$ T cells produce both Th1 and Th2 cytokines (4, 5, 43). The molecular mechanisms by which TGF-β controls T cell differentiation remain poorly understood. However, recent work has elucidated the redundant roles of the transcription factors Smad2 and Smad3 in TGF-β inhibition of Th1 differentiation (44, 45). The Smad signaling pathway has also been shown to mediate TGF-β inhibition of Th2 differentiation by inducing the transcription factor Sox4, which interferes with GATA-3 activity (46). In contrast to its role in inhibiting effector T cell differentiation, TGF-β has been shown to promote the Th17 lineage. The following sections specifically focus on TGF-β regulation of Th17 cells and cytotoxic T cells.

Th17 cell differentiation. A role for TGF-β in the induction of Th17 cells was established through in vitro differentiation assays and in vivo observations that mice with defective TGF-β signaling possessed fewer IL-17–producing T cells, whereas overexpression of *Tgfb1* led to an increase in Th17 cells (47–50). Mechanistically, TGF-β was suggested to promote the Th17 lineage by inhibiting the differentiation of other helper subsets, as stimulation with IL-6 alone was sufficient to induce IL-17 production in CD4$^+$ T cells lacking essential Th1 and Th2 molecules (51). In support of this, TGF-β–mediated suppression of *Gfi1* and *Eomesodermin* (*Eomes*; which promote Th2- and Th1-associated cytokines, respectively) was shown to enhance IL-17 production in CD4$^+$ T cells (52, 53). Indeed, although TGF-β stimulation induces RORγt expression in CD4$^+$ T cells, activation of STAT3 signaling promotes much stronger expression of this key Th17 transcription factor (54). Accordingly, in findings that paralleled studies of human Th17 differentiation, TGF-β was shown to be dispensable for murine Th17 differentiation, as stimulation with IL-23, IL-6, and IL-1β was sufficient to induce IL-17 production by CD4$^+$ T cells (55, 56).

Interestingly, a series of studies examining the ability of in vitro–differentiated Th17 cells to induce experimental autoimmune encephalomyelitis (EAE) suggests that TGF-β stimulation imprints a regulatory phenotype that renders the cells unable to cause disease. The initial study reported that myelin-reactive T cells isolated from myelin-immunized mice and expanded in the presence of IL-23, but not those cultured with TGF-β and IL-6, could induce EAE in a transfer model of disease (57). In this system, nonpathogenic, TGF-β–induced Th17 cells were associated with high levels of IL-10 production. Another group reported similar findings that Th17 cells induced by IL-23, IL-6, and IL-1β, but not those generated by TGF-β and IL-6, could induce EAE in a transfer model of disease (55). In contrast to TGF-β– and IL-6–induced Th17 cells, pathogenic Th17 cells were found to produce little IL-10 and express high levels of T-bet. Both studies used TGF-β1, the dominant isoform expressed in the immune system, to induce Th17 differentiation. Interestingly, a recent report suggests that an alternate TGF-β family member, TGF-β3, in conjunction with IL-6, promotes the in vitro differentiation of pathogenic, EAE-inducing Th17 cells by signaling through distinct Smads from TGF-β1 (58). Determining a definitive role for TGF-β3 in Th17 differentiation will require the study of T cell–specific *Tgfb3* knockouts; notably, however, the pathogenicity of TGF-β3–derived Th17 cells also required IL-23 signaling, as IL-23R–deficient cells did not induce disease. The reports that TGF-β1 inhibits the pathogenic activity of in vitro–generated Th17 cells in a transfer model of EAE conflicts with studies showing that

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T cell–derived *Tgfb1* is required for in vivo Th17 differentiation and disease development in a nontransfer model of EAE (59, 60). Whether these discrepancies result from differences between in vitro and in vivo Th17 differentiation, or between transfer and nontransfer models of EAE, remains to be determined.

Cytotoxic T cell differentiation. Observations of diminished in vitro $CD8^+$ T cell cytolytic activity in the presence of TGF-β provided early evidence for TGF-β–mediated inhibition of $CD8^+$ T cell responses (61). The inhibitory effects of TGF-β on $CD8^+$ T cell function were also indicated in vivo by studies showing that interfering with TGF-β signaling either by T cell–specific DNRII expression or by administration of a soluble TGF-βRII led to enhanced immune responses against transplantable tumors (62, 63). That TGF-β directly acts upon $CD8^+$ T cells to control their differentiation was definitively established by the finding that TGF-βRII–deficient $CD8^+$ T cells produce increased amounts of IFN-γ and cytolytic molecules (4, 5). Recent work in the TRAMP spontaneous prostate cancer model has provided additional evidence that TGF-β negatively regulates $CD8^+$ T cell responses. TRAMP mice with DNRII-expressing $CD8^+$ T cells exhibited tumor protection that correlated with decreased expression of the inhibitory marker programmed cell death-1 and increased expression of the cytolytic molecule granzyme B on tumor-infiltrating $CD8^+$ T cells (64). In a model of adoptive cell therapy in tumor-bearing TRAMP mice, TGF-βRII–deficient $CD8^+$ T cells demonstrated increased tumor infiltration and enhanced effector properties relative to their wild-type counterparts (65). However, studies in chronic viral and acute bacterial infections indicate the context-dependent nature of TGF-β control of $CD8^+$ T cell responses, as DNRII-expressing $CD8^+$ T cells showed enhanced effector properties only during a chronic viral infection (66, 67). In both infections, however, effector DNRII-expressing $CD8^+$ T cells underwent decreased apoptosis, suggesting that TGF-β may broadly promote effector $CD8^+$ T cell death. It will be important to understand the factors that dictate disparate effects of TGF-β signaling on $CD8^+$ T cell responses. Indeed, the complexity of TGF-β function has been emphasized by a recent study reporting that memory $CD8^+$ T cells expressing the DNRII, but not those with *Tgbr2* deletion, exhibit dysregulated homeostasis and undergo cellular transformation (68). This finding suggests that the dosage of TGF-β signaling may critically influence the resulting T cell phenotype.

TGF-β production and activation

TGF-β is synthesized in a latent form that must be activated to engage its receptors and initiate signaling. Distinct cellular sources can mediate the production and activation of TGF-β, providing another layer of complexity in the regulation and function of this pleiotropic cytokine.

T cell–derived TGF-β controls T cell tolerance. Although many different cell types produce TGF-β, the discovery that mice with T cell–specific *Tgfb1* deficiency develop autoimmunity and exhibit early mortality at ~6 mo of age demonstrated the critical contribution of T cell–derived TGF-β1 to immune tolerance (59). The tolerogenic function of T cell–produced TGF-β1 was corroborated by work showing that TRAMP mice with T cell–specific *Tgfb1* deletion exhibited a tumor protection phenotype that correlated with increased $CD8^+$ T cell effector function (64). It was subsequently shown that deletion of *Tgfb1* in activated $CD4^+$ T cells and Tregs, but not in Tregs or $CD8^+$ T cells alone, was sufficient for protection from B16-OVA melanoma metastasis, which correlated with increased $CD8^+$ T cell cytolytic activity (69). The identification of activated $CD4^+$ T cells as the essential source of TGF-β in this model indicated that paracrine TGF-β signaling plays an important role in imprinting tolerogenic $CD8^+$ T cell responses. Indeed, deletion of *Tgfb1* in activated $CD4^+$ T cells and Tregs was also found to be sufficient for tumor protection in the TRAMP spontaneous prostate cancer model. Notably, tumor-produced TGF-β was intact in both B16-OVA melanoma and TRAMP models, underscoring the importance of T cell–derived TGF-β in the regulation of T cell tolerance.

Effector T cell–derived TGF-β controls Th17 differentiation. In addition to controlling T cell tolerance, T cell–derived TGF-β1 has been found to play an essential role during in vivo Th17 differentiation. This was demonstrated by work showing that mice with T cell–specific deletion of *Tgfb1* harbored decreased frequencies of IL-17–producing T cells and exhibited protection from EAE (59). The CD4-Cre used in this system deletes *Tgfb1* in conventional $CD4^+$ and $CD8^+$ T cells as well as Tregs. Although Tregs could provide sufficient amounts of TGF-β1 to induce in vitro Th17 differentiation (50), Treg-derived TGF-β1 was not required for in vivo Th17 differentiation, as Th17 cell frequencies and EAE pathogenesis were comparable in wild-type mice and mice with Foxp3-Cre–mediated *Tgfb1* deletion (60). In contrast, deletion of *Tgfb1* using an Ox40-Cre that targets both Tregs and activated $CD4^+$ T cells led to a reduction in IL-17–producing $CD4^+$ T cells and EAE protection, identifying the encephalitogenic T cells themselves as critical TGF-β1 sources. Notably, among the $CD4^+$ Th cell subsets, Th17 cells were found to express the highest amounts of TGF-β1. These findings suggested that autocrine TGF-β1 signaling is essential for in vivo Th17 differentiation. This was confirmed by mixed bone marrow chimera experiments showing that *Tgfb1*-deficient T cells failed to produce IL-17 even in the presence of wild-type cells.

Activation of TGF-β. The latent complex of TGF-β consists of mature TGF-β that is noncovalently associated with the latency-associated protein (LAP). This latent form of TGF-β can be secreted or may associate with latent TGF-β–binding protein, which mediates deposition of the complex to the extracellular matrix. More recent work has shown that latent TGF-β can also associate with glycoprotein A repetitions predominant (GARP) protein, resulting in cell surface expression of TGF-β on Tregs and platelets (70). TGF-β can be activated from the membrane-bound complex of GARP and latent TGF-β, suggesting that GARP may function as an important regulator of TGF-β availability at cell surfaces (71). Release of TGF-β from its association with LAP is an essential step in TGF-β activation and function. Although multiple mechanisms, including thrombospondin and matrix metalloprotease activity, have been implicated in TGF-β activation, the major role of integrins in this process was demonstrated by studies showing that mice expressing a mutant *Tgfb1* allele that abolishes integrin binding to LAP phenocopy *Tgfb1*-deficient mice (72). The particular

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importance of myeloid cell integrin activity in TGF-β function was demonstrated by the observations that specific loss of α_v or β_8 integrins in myeloid or dendritic cells was sufficient to induce murine colitis (73, 74). Myeloid cell integrin-mediated activation of TGF-β has also been shown to play a critical role during in vivo Th17 differentiation, as mice with myeloid cell-specific α_v or β_8 integrin deficiency exhibit a reduction in Th17 cells and are protected from EAE (75, 76). The promotion of Th17 differentiation was specific to myeloid cell integrin activity, as T cell–specific integrin deficiency affected neither Th17 cell frequency nor EAE development. Thus, although effector T cells are critical sources of TGF-β during in vivo Th17 differentiation, a distinct innate cell type is required for TGF-β activation (60, 75). Notably, the same myeloid cell must present Ag and express integrins, suggesting tight spatiotemporal regulation of TGF-β activation during T cell stimulation (75, 76). Determining the precise innate cell types that express the $\alpha_v\beta_8$ integrins and how integrin expression is regulated during myeloid cell differentiation and activation will provide important insights into the complex biology of this cytokine.

Conclusions

Through its widespread regulation of T cells (Fig. 1), TGF-β ensures a diverse, self-tolerant, and functional T cell repertoire. The complex cellular network of TGF-β production and activation as well as the intricacy of TGF-β signaling pathways likely enable the pleiotropic functions of this cytokine. Yet, how this complicated regulation results in TGF-β control of T cells is incompletely understood. On a molecular level, although recent work has demonstrated the redundant roles of Smad2 and Smad3 in T cell regulation (44, 45), fundamental questions of how the Smad-dependent transcriptional program controls T cells remain unanswered. On a cellular level, the milder autoimmune phenotypes of mice with T cell–specific *Tgfb1* deficiency or dendritic cell–specific α_v or β_8 integrin deficiency versus mice with global defects in TGF-β production or activation indicate that other, unknown cell types are important for both of these processes. Although this review has focused on TGF-β control of T cells, the pervasive regulatory circuit of this cytokine affects other immune cells as well, and its regulation of non–T cell populations might also be critical for the prevention of autoimmunity. Expanding from a T cell–centric view of TGF-β regulation to include other cell types will provide increased insight into the mechanisms that govern TGF-β control of the immune system as well as potential therapeutic applications.

Disclosures

The authors have no financial conflicts of interest.

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