



Modulation of Regulatory T Cell Activity by TNF Receptor Type II-Targeting Pharmacological Agents

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There is now compelling evidence that tumor necrosis factor (TNF)–TNF receptor type II (TNFR2) interaction plays a decisive role in the activation, expansion, and phenotypical stability of suppressive CD4⁺Foxp3⁺ regulatory T cells (Tregs). In an effort to translate this basic research finding into a therapeutic benefit, a number of agonistic or antagonistic TNFR2-targeting biological agents with the capacity to activate or inhibit Treg activity have been developed and studied. Recent studies also show that thalidomide analogs, cyclophosphamide, and other small molecules are able to act on TNFR2, resulting in the elimination of TNFR2-expressing Tregs. In contrast, pharmacological agents, such as vitamin D3 and adalimumab, were reported to induce the expansion of Tregs by promoting the interaction of transmembrane TNF (tmTNF) with TNFR2. These studies clearly show that TNFR2-targeting pharmacological agents represent an effective approach to modulating the function of Tregs and thus may be useful in the treatment of major human diseases such as autoimmune disorders, graft-versus-host disease (GVHD), and cancer. In this review, we will summarize and discuss the latest progress in the study of TNFR2-targeting pharmacological agents and their therapeutic potential based on upregulation or downregulation of Treg activity.

Keywords: TNF receptor type II, regulatory T cells, TNF receptor type II agonists, TNF receptor type II antagonists, immunotherapy

INTRODUCTION

CD4⁺FoxP3⁺ regulatory T cells (Tregs) play an indispensable role in maintaining immunological homeostasis and inhibiting autoimmune responses, while they also represent a major cellular mechanism in immune evasion of tumors by dampening antitumor immune responses (1, 2). Consequently, Tregs have become important therapeutic target in the treatment of autoimmune diseases, graft-versus-host disease (GVHD), transplantation rejection, and cancer.

We (Xin Chen and Joost J. Oppenheim) previously reported that tumor necrosis factor (TNF)-alpha stimulates the activation and expansion of Tregs, and this effect of TNF is mediated by TNF receptor type II (TNFR2) (3). Moreover, we showed that the expression of TNFR2 correlated with suppressive function and phenotypical stability of Tregs (4–7). Our finding that TNF–TNFR2 interactions play a decisive role in Treg function is now supported by compelling evidence from both human Treg studies (8–24) and mouse Treg studies (25–40) by other groups. Some of these independent studies also clearly show that the Treg-stimulatory effect of TNF–TNFR2 pathway can be therapeutically harnessed for the treatment of major human diseases, including cancer and autoimmune disorders (10, 12, 14, 16, 18, 20, 23, 24).

TABLE 1 | TNF receptor type II (TNFR2)-targeting pharmacological agents.

Category	Class	Agent	Activity	Reference
TNFR2 agonists	Agonistic TNFR2 monoclonal antibodies (mAbs)	"TNFR2 antagonist"	<ul style="list-style-type: none"> Binds to and activates human TNFR2 Stimulates the activation and expansion of homogeneous and highly functional regulatory T cells (Tregs) isolated from normal donors and patients with type 1 diabetes (T1D) (<i>in vitro</i> assay) 	(10, 18)
		MR2-1 (isotype: IgG1)	<ul style="list-style-type: none"> Binds to and activates human TNFR2 Promotes the expansion of homogenous Foxp3⁺Helios⁺CD127^{low} Treg population with highly suppressive capacity (<i>in vitro</i> assay and in humanized mouse study) 	(20)
	Tumor necrosis factor (TNF) muteins	TNF07	<ul style="list-style-type: none"> Binds to and activates human TNFR2 Expands Foxp3⁺ Treg cells from normal donors (<i>in vitro</i> assay) Selectively induces the death of autoreactive CD8⁺ T cells from T1D patients (<i>in vitro</i> assay) 	(14)
		STAR2	<ul style="list-style-type: none"> Binds to and activates mouse TNFR2 Stimulates proliferative expansion of Foxp3⁺ Tregs (<i>in vitro</i> assay) Selectively activates and expands Foxp3⁺ Tregs in WT mice (<i>in vivo</i> assay) Markedly prolongs the survival and decreases the severity of graft-versus-host disease (GVHD) (<i>in vivo</i> assay) 	(38)
TNC-scTNF(R2)		<ul style="list-style-type: none"> Binds to and activates human TNFR2 Protects TNFR2-expressing oligodendrocyte progenitor cells from death induced by oxidative stress (<i>in vitro</i> assay) Unknown effect on human Tregs 	(41)	
Anti-TNF mAbs	Adalimumab	EHD2-scTNFR2	<ul style="list-style-type: none"> Binds to and activates mouse TNFR2 Inhibits neuroinflammation and promotes neuronal survival in a mouse model of neurodegeneration in combination with a TNFR1 antagonist (<i>in vivo</i> assay) Unknown effect on mouse Tregs 	(42)
		Infliximab	<ul style="list-style-type: none"> A therapeutic humanized mAb binding to both soluble TNF (sTNF) and transmembrane TNF (tmTNF) Increases expression of tmTNF on monocytes from rheumatoid arthritis (RA) patients (<i>in vitro</i> assay) Promotes the binding of tmTNF (expressed on monocytes) to TNFR2 (expressed by Tregs of RA patients), resulting in selective activation and proliferation of Tregs (<i>in vitro</i> assay) 	(16)
Small molecule compounds	Vitamin D3	Infliximab	<ul style="list-style-type: none"> A therapeutic humanized mAb against TNF-α Increases the suppressive function of Tregs in autoimmune patients, at least partially caused by the elevated levels of TNF (<i>in vivo</i> assay) 	(4, 15, 22)
		Vitamin D3	<ul style="list-style-type: none"> VD3-DCs induces induced Tregs (iTregs) through the interaction of tmTNF expressed by VD3-DCs and TNFR2 expressed by Tregs (<i>in vitro</i> assay) 	(26)
TNFR2 antagonists	Antagonistic TNFR2 mAbs	"TNFR2 antagonist"	<ul style="list-style-type: none"> Blocks the binding of TNF to human TNFR2 Markedly inhibits the expansion of Tregs and reduces the suppressive capacity of Tregs (<i>in vitro</i> assay) 	(10)
		Dominant anti-human TNFR2 antagonistic Abs	<ul style="list-style-type: none"> Block the binding of TNF to human TNFR2 and hamper TNFR2 signaling activation Inhibit TNF-induced expansion of human Tregs (<i>in vitro</i> assay) Induce the death of Tregs, especially those isolated from ovarian cancer tissue (<i>in vitro</i> assay) Induce the death of TNFR2-expressing OVCAR3 tumor cells (<i>in vitro</i> assay) 	(23)

(Continued)

TABLE 1 | Continued

Category	Class	Agent	Activity	Reference
	Small molecule compounds	Thalidomide and its analogs	<ul style="list-style-type: none"> Inhibit TNF synthesis Inhibit the surface expression of TNFR2 on T cells (<i>in vitro</i> assay) Reduce the number and function of Tregs and TNFR2 expression on Tregs in patients with leukemia (<i>in vivo</i> assay) Increase the number of Tregs in patients with multiple myeloma (MM) (<i>in vivo</i> assay) 	(13, 62, 63, 65, 67, 110)
		Panobinostat	<ul style="list-style-type: none"> Reduces the expression of Foxp3 and inhibit the suppressive function of Tregs at low doses (<i>in vitro</i> assay) Reduces the proportions of TNFR2⁺ Tregs in the blood and bone marrow of acute myeloid leukemia (AML) patients in combination with azacitidine (<i>in vivo</i> assay) 	(12, 70)
		Cyclophosphamide	<ul style="list-style-type: none"> Selectively depletes TNFR2^{hi} Tregs population in a mouse model of mesothelioma (<i>in vivo</i> assay) 	(74)
		Triptolide	<ul style="list-style-type: none"> Reduces TNF and TNFR2 expression in colon of colitis mice (<i>in vivo</i> assay) Reduces the number of Tregs and inhibits tumor growth in melanoma-bearing mice (<i>in vivo</i> assay) 	(76, 77)

To translate this basic research finding into therapeutic benefit, a number of agonistic or antagonistic TNFR2-targeting biological agents with the capacity to upregulate or downregulate Treg activity have been developed. Recent study also revealed that some small molecule compounds can suppress TNFR2 expression or eliminate TNFR2-expressing Tregs. Some pharmacological agents were found to induce Tregs by promoting interaction of transmembrane TNF (tmTNF) with TNFR2. In this brief review, recent reports of TNFR2-targeting pharmacological agents with the capacity to upregulate or downregulate Treg activity were reviewed, analyzed, and discussed (Table 1).

TNFR2 AGONISTIC BIOLOGICAL AGENTS

Faustman's group has screened a panel of monoclonal antibodies (mAbs) against human TNFR2 generated from her own lab or purchased from commercial sources. They identified a potent agonistic TNFR2 mAb which was designated as "TNFR2 agonist" in their study. In the presence of IL-2, "TNFR2 agonist" potentially stimulated the expansion of Foxp3⁺ Tregs present in cultures of CD4 cells, accompanied by the upregulation of TNF, TRAF2, TRAF3, BIRC3 (cIAP2), and Foxp3 mRNA expression (10). Furthermore, this property of the "TNFR2 agonist" was harnessed to generate highly homogenous Foxp3⁺ Tregs. To this end, MACS-purified CD4⁺CD25⁺ cells were cultured under standard *in vitro* human Treg expansion conditions (anti-CD3 Ab, anti-CD28 Ab, IL-2, and rapamycin), with or without the "TNFR2 agonist." Expanded Tregs in the presence of "TNFR2 agonist" expressed markedly higher levels of Foxp3 and other characteristic Treg markers, and possessed more potent suppressive capacity (10). More recently, Faustman's group examined the effect of such "TNFR2 agonist" on the activation and expansion of Tregs isolated from patients with type 1 diabetes (T1D) (18). The results show that *in vitro* treatment with "TNFR2 agonist" stimulated the activation of T1D Tregs which initially showed a resting phenotype. Furthermore, under the aforementioned standard Treg expansion culture condition, "TNFR2 agonist" promoted the homogenous expansion of Tregs isolated from T1D patients by magnetic beads (18). "TNFR2 agonist"-expanded T1D Tregs were more potent in the inhibition of autologous CD8⁺ T cells (18). A similar result was obtained by using MR2-1, a commercially available agonistic human TNFR2 mAb (mouse IgG₁) by another group (He/Joosten and colleagues) (20). In this study, low purity MACS-isolated human Tregs were expanded with the aforementioned standard protocol. The treatment with MR2-1 resulted in the generation of more homogenous Foxp3⁺Helios⁺CD127^{low} Tregs. The phenotype of resultant Treg cells remained stable, even in the pro-inflammatory environment. Importantly, Tregs expanded with MR2-1 maintained highly suppressive activity in a humanized mouse model (20). Thus, TNFR2 agonists can facilitate *ex vivo* expansion of Treg cells from less pure population for Treg-based immunotherapy.

Prompted by the potential therapeutic effect on autoimmune diseases, Faustman's group also generated soluble TNF (sTNF) muteins with TNFR2 agonistic effect, designated S95C/G148C or TNF07 (14). This stable TNF trimer, TNF07 double mutant, functioned as a TNFR2 agonist. It could trigger a strong TNFR2

signaling, with the capacity to expand Foxp3⁺ Treg cells and to selectively induce the death of autoreactive CD8⁺ T cells isolated from T1D patients (14).

Chopra/Beilhack and colleagues developed a novel nonameric TNFR2-specific variant of mouse TNF (STAR2), which was a selective agonist of mouse TNFR2 and had no capacity to bind to TNFR1 (38). STAR2 had *in vitro* and *in vivo* activity to stimulate the proliferation of Tregs in a TNFR2-dependent and IL-2-independent manner. Furthermore, pretreatment with STAR2 before allogeneic hematopoietic stem cell transplantation (allo-HCT) markedly prolonged the survival and decreased the severity of GVHD, in TNFR2- and Treg-dependent manner. A human TNFR2-specific STAR2 equivalent agonist also potently stimulated the expansion of Foxp3⁺ Tregs from healthy donors *in vitro* (38).

A number of TNFR2-targeting agents, such as TNC-scTNF(R2) (a human TNFR2 selective agonist) (41) and EHD2-scTNFR2 (a mouse TNFR2 selective agonist) (42), were developed to examine their protective effect on neurodegeneration. It would be interesting to ask if their neuroprotective effect is attributable to their capacity to activate and expand Tregs, and if they have beneficial effect in the inhibition of autoimmune diseases.

It was shown recently that TROS, a nanobody-based selective inhibitor of TNFR1, was able to inhibit mouse experimental autoimmune encephalomyelitis (EAE) and this effect is attributable to the diversion of TNF to interact with TNFR2 (43). TNFR2 is also expressed by oligodendrocytes or astrocytes, with neuroprotective function through tmTNF-TNFR2 signaling to promote CNS cells differentiation and remyelination, and such effect of TNFR2 signaling was based on its directly action on the cells in CNS (44–46). Therefore, selectively blocking TNFR1, thus favoring TNFR2, may represent another strategy to stimulate TNFR2⁺ Tregs in the treatment of autoimmune diseases and GVHD.

TNFR2 ANTAGONISTIC BIOLOGICAL AGENTS

In addition to a TNFR2 agonist, Faustman's group also identified a potent mAb antagonist of human TNFR2, designated as "TNFR2 antagonist" in their study (10). In the standard Treg expansion culture condition, this "TNFR2 antagonist" markedly inhibited the expansion of Tregs and reduced the suppressive capacity of Tregs (10). More recently, Torrey/Faustman and colleague developed two potent dominant anti-human TNFR2 antagonistic Abs that outcompeted TNF, the natural agonist of TNFR2, and inhibited TNF-induced *in vitro* expansion of human Tregs (23). These TNFR2 antagonists specifically bound to TNFR2 through F(ab) region, independent of Fc region or crosslinking of antibodies. Through binding to the antiparallel dimers of TNFR2 protein, the TNFR2 antagonists blocked the binding of TNF to TNFR2. Consequently, they inhibited TNF-triggered activation of nuclear factor- κ B (NF- κ B) pathways in Tregs, and suppressed conversion of tmTNFR2 to sTNFR2. These two TNFR2 antagonists could induce the death of Tregs *in vitro*. Interestingly, Tregs isolated from ovarian cancer tissues were more sensitive to TNFR2 antagonist-induced cell death (23), presumably attributable to the

higher levels of TNFR2 expression on tumor-infiltrating Tregs (4). TNFR2 is also expressed on the surface of OVCAR3, an ovarian cancer cell line. Intriguingly, TNFR2 antagonists could also induce the death of OVCAR3 tumor cells (23). Thus, this *in vitro* evidence strongly supports the idea that TNFR2 antagonists may represent novel cancer therapeutics by simultaneously targeting tumor-infiltrating Tregs and tumor cells.

Progranulin (PGRN), a glycosylated protein, has immunosuppressive and anti-inflammatory activity (47–49), presumably due to its capacity to promote the induction of induced Tregs (iTregs), as shown in an *in vitro* study (50). Progranulin was initially reported as an endogenous TNFR2 antagonist (51). However, controversial results were reported (52, 53) and thus further study is needed to clarify its effect on TNFR2.

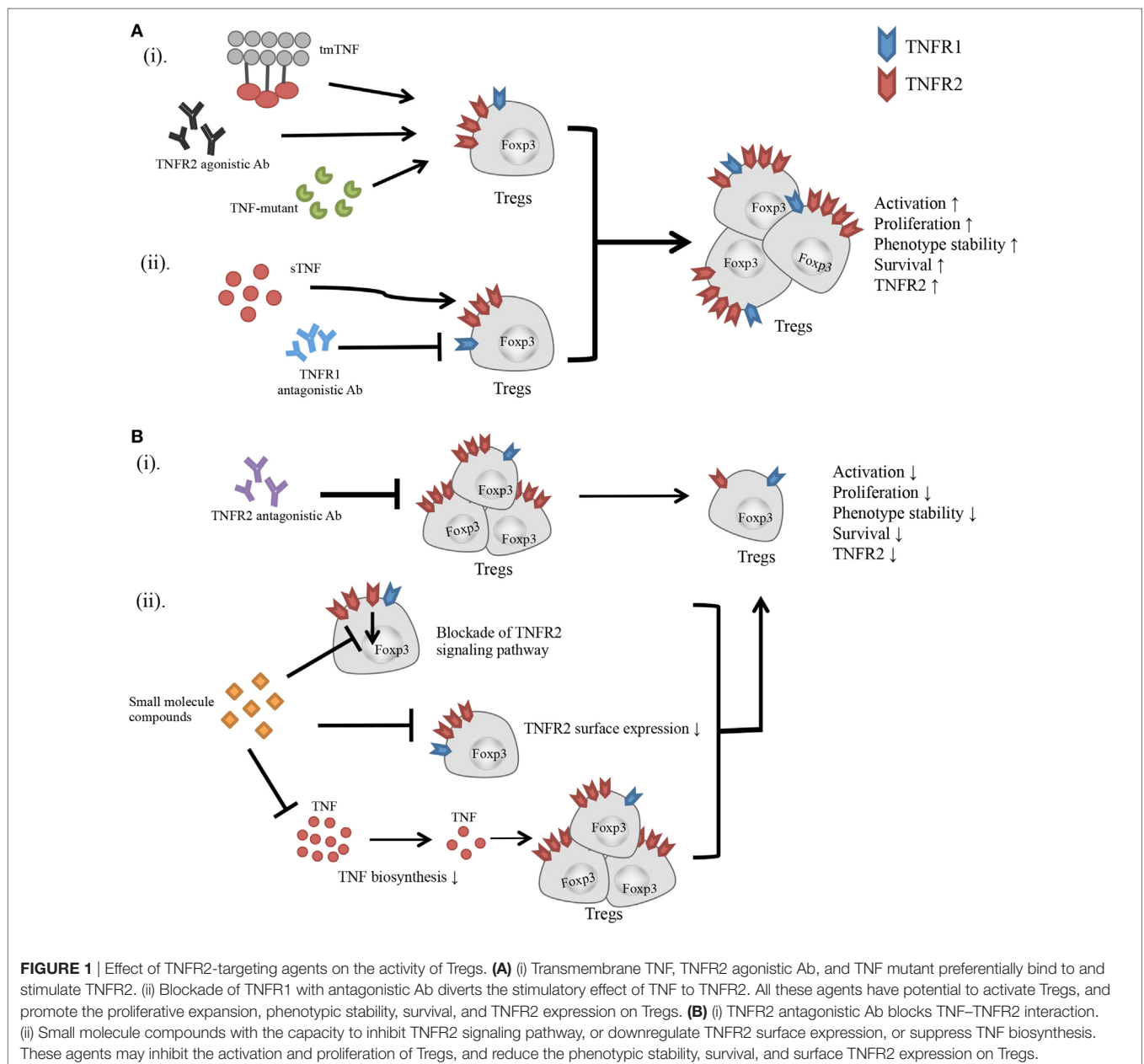
SMALL MOLECULE TNFR2 INHIBITORS

Thalidomide is a synthetic small molecule glutamic acid derivative (54) that was initially developed for alleviation of morning sickness of pregnant women in Europe several decades ago (55). It was withdrawn from the market because it caused developmental defects in newborns (55). The interest in using this compound as a therapeutic agent reawakened recently, due to its suggested effect in the treatment of erythema nodosum leprosum (ENL) (56, 57). This led to the discovery of immunomodulatory and anti-inflammatory properties of thalidomide and to clinical trials of thalidomide and its analogs in various malignancies (54). Thalidomide and its structural analogs (lenalidomide and pomalidomide) are now classified as immunomodulatory drugs (IMiDs) (54). It has been well established that thalidomide and its analogs are able to inhibit TNF protein synthesis through downregulation of NF- κ B, destruction of TNF mRNA, and targeting reactive oxygen species and α 1-acid glycoprotein (58–61). Thalidomide and its analogs also have the capacity to inhibit the surface expression of TNFR2 on T cells without reducing the expression of total TNFR2 protein (62), which is associated with the inhibition of intracellular TNFR2 transport to the cell surface (13). Giannopoulos et al. showed that, in patients with chronic lymphocytic leukemia, thalidomide treatment reduced the number and function of Tregs (63, 64), presumably by blockade of TNF-TNFR2 interaction. Moreover, Plebanski's group reported that, in acute myeloid leukemia (AML) patients, combination therapy with lenalidomide and a demethylating agent, azacitidine, downregulated TNFR2 expression on CD4 T cells and reduced the number of TNFR2⁺ Tregs, resulting in enhanced effector immune function (13). However, it was reported that treatment with thalidomide and its analog actually increased the number of Tregs in patients with multiple myeloma (MM) (65, 66), which may be attributable to the elevated serum levels of TNF after treatment (62, 66). Furthermore, thalidomide was reported to promote *de novo* generation of iTregs (67), which is consistent with current understanding of responses of iTreg to TNF-TNFR2 stimulation (29, 68). Thus, the effect of thalidomide on TNFR2⁺ Tregs is likely to be disease- and condition-specific, which should be clarified by future study.

Histone deacetylase inhibitor panobinostat is effective in the treatment of MM in combination with bortezomib and dexamethasone (69). A recent study found that low doses of

panobinostat could reduce the expression of Foxp3 and inhibit the suppressive function of Tregs (70). Furthermore, Govindaraj et al. reported that the combination treatment with panobinostat and azacitidine reduced the proportions of TNFR2⁺ Tregs in the blood and bone marrow of AML patients (12). One of the mechanisms may be the disruption of the AML bone marrow niche by panobinostat and azacitidine, resulting in reduced blast cell levels and preventing Treg induction by blast cells (12). The reduction of TNFR2⁺ Tregs and consequently increase of IFN γ and IL-2 production by effector T cells (Teffs) is attributable to the clinical beneficial effect of patients with AML (12). This study indicates that epigenetic therapeutics may represent a strategy to eliminate TNFR2⁺ Treg activity and to enhance antitumor immune responses.

Cyclophosphamide (CY) is a DNA alkylating agent which is commonly used as a cytotoxic chemotherapy in cancer treatment (71). CY at low dosages can inhibit immunosuppressive function of Tregs (72), and a single dose of CY depletes the maximally suppressive Tregs in PROb colon cancer bearing mice, resulting in the activation of antitumor immune responses (73). Moreover, van der Most et al. reported that, in a mouse model of mesothelioma, CY treatment depleted TNFR2^{hi} Tregs (74). This effect of CY was based on its capacity to induce the death of replicating Tregs which co-express TNFR2 and Ki-67 (4, 74). Furthermore, CY in combination with etanercept, a therapeutic TNF antagonist, markedly inhibited the growth of established CT26 tumor in mice, by eliminating TNFR2-expressing Treg activity through blockade of TNF–TNFR2 interaction (75).



Triptolide (TPT), an immunosuppressive compound isolated from Chinese herb *Tripterygium wilfordii* Hook F., was reported to inhibit TNF as well as TNFR2 expression in the colon of mouse colitis model (76). TPT was also reported to decrease the number of Tregs and consequently inhibited the growth of mouse tumor (77). Thus, it would be interesting to investigate if TPT and other naturally occurring compounds have the capacity to downregulate Treg activity by blockade of TNF–TNFR2 interaction.

PHARMACOLOGICAL AGENTS THAT PROMOTE THE INTERACTION OF tmTNF AND TNFR2

TNF binds and signals through two structurally related functionally distinct receptors: TNFR1 and TNFR2 (78). Once synthesized, TNF is expressed initially as a cell surface type II polypeptide consisting of 233 amino acid residues (26 kDa). Transmembrane TNF is then cleaved by TNF- α converting enzyme into a sTNF consisting of 157 amino acid residues (17 kDa) (79). Soluble TNF predominantly binds and activates TNFR1, while tmTNF preferentially binds and activates TNFR2 (80). Therefore, agents which have the capacity to enhance the expression of tmTNF or promote the interaction of tmTNF and TNFR2 may also selectively activate and expand Tregs. This is exemplified by a recent study reported by Nguyen/Ehrenstein showing the paradoxical effect of adalimumab in the expansion of Tregs (16). Adalimumab is a therapeutic anti-TNF mAb which is effective in the treatment of rheumatoid arthritis (RA) and other autoimmune diseases (81). This Ab was developed to bind to both sTNF and tmTNF, aiming to block the interaction of TNF with its receptors (82). It was reported that adalimumab treatment increases the number of Tregs in RA patients (83). A recent *in vitro* study found that adalimumab bound to tmTNF expressed by monocytes from RA patients. This resulted in the upregulation of tmTNF expression, consisting with *in vivo* observations that adalimumab treatment enhanced TNF expression by monocytes from RA patients (16). Furthermore, adalimumab promoted the binding of tmTNF expressed by monocytes to TNFR2 expressed by Tregs of RA patients, consequently enhanced the activation and proliferation of Tregs (16). This study suggests that targeting of tmTNF–TNFR2 interaction may represent a novel strategy in the treatment of autoimmune diseases, especially in those patients that do not respond to conventional anti-TNF treatment, by mobilization of TNFR2⁺ Tregs (84). Coincidentally, these findings also clarify why adalimumab is more effective in the treatment of Crohn's disease (85), than etanercept which merely inhibits the effect of sTNF without the concomitant stimulation of Tregs (85, 86).

Infliximab (Remicade) is a therapeutic chimeric mAb against TNF used in the treatment of autoimmune diseases (87). A recent study shows that, in patients with sarcoidosis, surface expression of TNFR2 on CD4⁺CD25^{hi} “Tregs” was higher in responders to therapy, as compared to those non-responders (22). Since TNFR2 expression is associated with suppressive function of Tregs (4, 15), this study suggests that infliximab treatment may also increase the suppressive function of Tregs in autoimmune patients.

It was reported that tolerogenic dendritic cells (DCs), designated as VD3-DCs, were induced by the treatment with 1 α , 25-dihydroxyvitamin D3 (VD3). Such DCs expressed high levels of TNF and PD-L1 upon LPS stimulation and were able to induce functionally suppressive Tregs (88). A subsequent study by the same group (Kleijwegt/Roep and colleagues) found that VD3-DCs expressed high levels of tmTNF. Furthermore, induction of Ag-specific Tregs by VD3-DCs depended on the interaction of tmTNF expressed by VD3-DCs and TNFR2 expressed by Tregs, since blockade of binding of tmTNF to TNFR2 abrogated the induction of suppressive function of Tregs (26). In this study, Tregs induced by VD3-DCs were converted from naïve CD4 T cells (26). Thus, the possibility that VD3-DCs can also promote the activation and expansion of naturally occurring Tregs (nTregs) in a tmTNF–TNFR2 dependent manner, especially in the physiologically relevant *in vivo* settings, should be addressed in a future study. Furthermore, since CD8⁺Foxp3⁺ Tregs also expressed high levels of TNFR2 on their surface and TNF signaling is required for the generation of CD8⁺Foxp3⁺ Tregs (89), it would be interesting to investigate if they can be generated or expanded by tmTNF-expressing VD3-DCs.

CONCLUSION

Although the first of the TNFR2 inhibitors identified was thalidomide (62), recent research actually focused on the development of TNFR2-targeting biological agents. This may be because the difficulty to block TNF–TNFR interaction with a small molecule, due to the large contact surface area (90), and due to the apparent advantage of biological therapeutics, such as high target specificity, well-understood mechanism and minimal toxicity (91, 92). Nevertheless, cell-permeable small molecules may also effectively block TNFR2 signaling pathways, and consequently inhibit Treg activity induced by TNF–TNFR2 interaction. So far, three signaling pathways of TNFR2 in T lymphocytes, e.g., IKK/NF κ B, MAPK (Erk1/2, p38, JNK), and PI3K/Akt pathways, have been reported (93–95). The effect of small molecule inhibitors specific for major components of these pathways on Treg activity should be investigated. Thoroughly understanding of TNFR2 signaling pathways in Tregs, especially those different from Teffs, is a key to identify or design selective Treg inhibitors and thus merits future study. Moreover, it has been shown that TNFR2-specific TNF muteins have the capacity to activate and expand Tregs (38). Since LT α homotrimer can also bind to TNFR2 (96), it would be interesting to investigate if TNFR2-specific mutant LT α have the capacity to preferentially activate Tregs.

In addition to being constitutively and predominantly expressed by highly suppressive Tregs (4), TNFR2 can also be induced and upregulated on CD4⁺Foxp3⁺ Tregs upon TCR stimulation (28, 97). However, the level of TNFR2 expressed by Tregs is much lower than its expression on T cells (6, 9, 23, 28). This may explain why TNFR2 antibody mimetics preferentially bind to Tregs (21). Nevertheless, TNFR2-targeting agents on the function of Tregs should be carefully evaluated in the future study. Furthermore, in addition to T cells, TNFR2 is also expressed by other cell types, such as endothelial cells (98), microglia and selected neuronal subtypes (99, 100), oligodendrocytes (101),

cardiac myocytes (102), and thymocytes (103). Since those TNFR2-expressing cells can also respond to TNFR2-targeting therapeutics, the off-target effect and safety of TNFR2 agonist and antagonist should be carefully evaluated.

Current experimental evidence suggest that TNFR2-targeting agents preferentially act on Tregs, and consequently promote or inhibit immune responses by downregulating or upregulating TNFR2⁺ Treg activity (Figure 1). However, this idea has to be confirmed by more physiologically relevant *in vivo* studies. TNFR2 is also reported to play a key role in the accumulation and immunosuppressive function of myeloid-derived suppressive cells (MDSCs) (34, 104, 105) and mesenchymal stem cells (MSCs) (106, 107). Since these cells exert their immunosuppressive function in a collaborative manner with Tregs (108, 109), the effect of a TNFR2 agonist or antagonist may have a greater effect on the modulation of immune responses, by acting on multiple components of the immunosuppressive network.

Taken together, recent studies regarding TNFR2-targeting agents not only further confirmed and substantiated the concept that TNFR2 signaling plays a decisive role in the activation and expansion of Tregs but they also clearly indicate that TNFR2-targeting pharmacological agents have great potential in the

treatment of major human diseases and deserve further research and development.

AUTHOR CONTRIBUTIONS

HZ, RL, and XC drafted the manuscript. HZ, RL, HH, YH, and XC approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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