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Chapter

TNFR2 and Regulatory T Cells: Potential Immune Checkpoint Target in Cancer Immunotherapy

Xuehui He and Xinhui Wang

Abstract

TNF has both proinflammatory and antiinflammatory effects. It binds to two structurally related but functionally distinct receptors TNFR1 and TNFR2. Unlike TNFR1 that is ubiquitously expressed, TNFR2 expression is more limited to myeloid and lymphoid cell lineages including a fraction of regulatory T cells (Treg). In general, TNFR1 is responsible for TNF-mediated cell apoptosis and death, and mostly induces proinflammatory reactions. However, TNFR2 mainly leads to functions related to cell survival and immune suppression. Treg play an indispensable role in maintaining immunological self-tolerance and restraining excessive immune reactions deleterious to the host. Impaired Treg-mediated immune regulation has been observed in various autoimmune diseases as well as in cancers. Therefore, Treg might provide an ideal therapeutic target for diseases where the immune balance is impaired and could benefit from the regulation of Treg properties. TNFR2 is highly expressed on Treg in mice and in humans, and TNFR2+ Treg reveal the most potent suppressive capacity. TNF-TNFR2 ligation benefits Treg proliferation, although the effect on Treg suppressive function remains controversial. Here, we will describe in detail the TNF-mediated regulation of Treg and the potential clinical applications in cancer immunotherapy as well as in autoimmune diseases, with the focus on human Treg subsets.

Keywords: TNF, TNF receptor 2, regulatory T cells, immunotherapy, autoimmune disease, cancer immunotherapy

1. Introduction

CD4+FOXP3+ regulatory T cells (Treg) have an indispensable role in maintaining immune homeostasis and immune tolerance. They control unwanted immune responses that are involved in the regulation of immune tolerance to self as well as to foreign antigens. Loss-of-function mutation in *FOXP3* locus, a gene encoding Treg lineage transcription factor FOXP3, leads to multiorgan associated autoimmunity. Abnormal numbers of Treg and/or impaired suppressive function of Treg are often found in various autoimmune diseases like type 1 diabetes (T1D) [1], multiple sclerosis (MS) [2], rheumatoid arthritis (RA) [3], psoriasis [4–6], and systemic lupus erythematosus (SLE) [7–9]. On the other hand, tumor-infiltrating Treg generally show potent suppressive functions, indicating that they regulate tumor-specific immune responses and might help tumor immune escape [10]. It seems

logical to use Treg as a therapeutic target for diseases where the immune balance is impaired and could benefit from the regulation of Treg properties. Nevertheless, due to the intrinsic properties of Treg, i.e. heterogeneity and plasticity, several key questions need to be clarified before making Treg an ideal candidate for clinical applications.

Tumor necrosis factor (TNF) is initially expressed on cell surface as a membrane bound cytokine (mTNF), which can be cleaved by a metalloprotease TNF converting enzyme (TACE) to generate soluble form of TNF (sTNF) [11]. TNF binds to receptors, TNF receptor 1 (TNFR1) and 2 (TNFR2). In contrast to TNFR1, TNFR2 expression is restricted in certain cell types including lymphocytes [12]. TNF-TNFR1 interaction mostly induces proinflammatory reactions, whereas TNFR2 generally leads to the suppressive function of TNF [13]. It is known that TNFR2 is constitutively expressed on both murine and human Treg, and TNFR2+ Treg are the most suppressive Treg subpopulation [14–17]. The effect of TNF on Treg suppressor function remains controversial. In this chapter, we will describe in detail the TNF-mediated signal transduction pathways, its effect on Treg cells, and the potential clinical applications in various immunopathologies.

2. Regulatory T cells and its plasticity

Treg exert their function in primary and secondary lymphoid organs and non-lymphoid tissues. FOXP3, as the lineage transcription factor of Treg, facilitates Treg thymic development by stabilizing its own expression and inhibiting transcription factors needed for the development of other helper T-cell (Th) lineages like T-bet for Th1, GATA3 for Th2, and RORγt for Th17 cells [18]. Next to FOXP3, Treg constitutively express a high level of the IL-2 receptor α chain (CD25) and a low level of the IL-7 receptor α chain (CD127) compared to human activated non-Treg. The combination of CD4+, CD25high, and CD127low has been used to isolate Treg for functional studies and for adoptive immunotherapy [19]. However, no unique Treg marker has been identified so far, although many molecules are proposed. These Treg-related cell markers include CD27 [20], CD62L [21], CTLA4 (cytotoxic T-lymphocyte-associated protein) [22], CD39 and CD73 ectoenzymes [23], Helios [24], Neuropilin-1 [25], HLA-DR [26], and the most recently identified combination of TIGIT and FcRL3, which results in the identification of human Helios+ memory Treg [27].

Compelling evidence indicates that both mouse and human Treg consist of various subpopulations and have a more or less plastic phenotype depending on the microenvironment they are in [28]. Based on the site of Treg generation, two major Treg subsets are classified, namely, thymus-derived Treg (tTreg) that develop in the thymus from CD4 single positive thymocytes which in general display high-affinity self-reactive T-cell receptors (TCRs), and peripherally induced Treg (pTreg) which emerge in the periphery from conventional CD4+ T lymphocytes (Tconv) in response to environmental antigens and tolerogenic stimuli. Studies in mice have shown that pTreg and tTreg are both required for full protection against colitis and lymphoproliferative disease [29, 30], indicating that these two Treg subsets play distinct roles in protecting against immunopathology. However, the relative contribution of tTreg and pTreg in human immune tolerance remains a major unresolved issue, partially due to the lack of specific markers to definitively distinguish them. In fact, the transcription factor Helios was the first marker proposed to distinguish both mice and human tTreg from pTreg [31]. However, this has been disputed by studies showing that Helios can also be expressed by activated Tconv [32] and by pTreg upon in vitro and in vivo stimulation [33], precluding its

use as tTreg-specific marker. Another cell surface marker that has been proposed to harbor the specificity necessary to distinguish between murine tTreg and pTreg is the coreceptor Neuropilin-1 [25]. Unfortunately, human Treg do not uniquely express Neuropilin-1 [34].

3. TNF/TNFR signaling pathways

TNF is firstly discovered as an inflammatory cytokine that is induced by the endotoxin [35]. Various immune cells produce TNF including macrophages, monocytes, dendritic cells, B cells, activated natural killer cells, and activated T cells. TNF is initially expressed on the cell surface as a trimeric type II transmembrane protein mTNF, which is then cleaved by the metalloproteinase TACE (also known as ADAM17) and released as soluble extracellular sTNF [36]. Both forms of TNF are present as bioactive homotrimers. There exist two structurally related but functionally distinct receptors, TNFR1 (p55) and TNFR2 (p75). TNFR1 is ubiquitously expressed on most mammalian cell types, and it binds to mTNF as well as sTNF, whereas TNFR2 expression is restricted to immune cells, neurons, and endothelial cells. TNFR2 binds with higher affinity to mTNF than sTNF compared to TNFR1.

TNFR1 and TNFR2 share the similar extracellular TNF-binding motifs but differ in their intracellular domains. Both receptors lack intrinsic enzyme activity; thus, upon the ligand binding, they need to recruit the cytosolic proteins to initiate the intracellular signal transduction. Specifically, TNFR1 contains a homologous intracellular region called "death domain", which preferentially interacts with the adaptor protein named TNFR1-associated death-domain (TRADD) protein [37]. TRADD further recruits another two adaptor proteins, receptor interacting protein kinase 1 (RIPK1) and TNFR-associated factor (TRAF) 2, thus forming an enzymatic complex signalosome, which is also known as signaling complex 1. One of the main targets of the complex 1 is the enzyme complex called IkB kinase (IKK). Phosphorylation of IKK in turn leads to the canonical activation of the transcription factor NFkB as well as members of the family of MAPKs such as c-jun kinase (JNK) and p38 MAPK. The TRADD containing signaling complex 1 may further be converted to a death-inducing signaling complex, so-called complex 2, by adaptor protein Fas-associated protein with death domain (FADD). The complex 2 is able to further initiate downstream caspase cascades, thus inducing cell apoptosis and cell death [37].

The pathways induced by TNFR2 are slightly different from TNFR1. Due to the lack of death domain, TNFR2 is unable to recruit TRADD protein, but it can directly interact with TRAF2 [38]. In contrast to TNFR1 that drives apoptosis and cell death, TNFR2 induces the noncanonical activation of NFκB via the activation of the NFκB-inducing kinase (NIK), which further leads to the phosphorylation of IKKα and the processing of p100, a crucial step in the nuclear translocation of p52/RelB [38, 39]. Interestingly, TRAF2 binding to TNFR2 is considerably weaker than its binding to TRADD protein. Upon binding to TRAF2, TNFR2 could also recruit cIAP1/2 proteins [39] that are involved in the TNFR1-mediated NFκB activation, indicating that there exists a crosstalk between TNFR1 and TNFR2 pathways. Another interesting adaptor protein called endothelial/epithelial protein tyrosine kinase (Etk) interacts with the C-terminal domain of TNFR2 in a ligand-independent manner [40]. TNFR2-mediated Etk phosphorylation is able to partially activate the growth factor receptor VEGFR2, which in turn results in the activation of PI3K/Akt pathway and cell survival.

A number of proteins are essential for the negative regulation of the TNF-TNFR pathways. A20, also named as TNF alpha-induced protein 3, is one of the most

studied negative regulatory proteins. A20 is an ubiquitin editing enzyme. It limits NFκB signaling after activation by TNF [41]. Consistent with this, A20-deficient mice are hypersensitive to TNF exposure and die perinatally because of severe inflammation and multiorgan failure [42]. Intriguingly, A20 is recently shown to regulate the de novo generation of tTreg in a cell-intrinsic manner, while the suppressor function of A20-deficient Treg is unchanged in vitro [43].

4. Effect of TNFR2 on Treg

Although TNFR1 expression is not different between Treg and non-Treg cells, human Treg constitutively express high levels of TNFR2 compared to CD25- Tconv. Moreover, TNFR2+ Treg reveal the most potent suppressive capacity [14, 44]. The effect of TNF on Treg suppressor function remains controversial. Several groups including ours demonstrated that sTNF preserved or even increased FOXP3 expression as well as Treg suppressive capacity in both mice and humans [15, 45–47]. The TNF-TNFR2 is crucial for sustaining FOXP3 expression and maintaining the stability of murine Treg in an inflammatory environment [44]. A similar phenomenon is also observed for human Treg in vitro [48]. There is also evidence for the negative effects of TNF on Treg function. Studies show that TNF impairs Treg function by reducing FOXP3 expression or enhancing its dephosphorylation [47, 49]. In clinical practices, RA patients responding to anti-TNF antibody adalimumab showed an increased percentage of FOXP3 + cells as well as the restored regulatory function [50]. It should be noted that the nature of the TNFR2 antibodies used in these studies was likely different (agonistic versus antagonistic) [46]. Recent studies highlight that TNFR2 agonisms and antagonisms might regulate the phenotype and the suppressor function of Treg in a complete different way [46].

TNF priming induces the proliferation and activation of Treg in vitro [15, 51] as well as in vivo via TNFR2 in an acute mouse GvHD model [52]. Our group have found that stimulation of human Treg with a TNFR2-agonist antibody preserved a stable Treg phenotype and function after ex vivo expansion [48]. Using TNFR2 agonist only was enough to prevent the loss of FOXP3 expression, whereas the sustained hypomethylation of TSDR (Treg-specific demethylated region) of *FOXP3* gene locus required both rapamycin and TNFR2 agonist, suggesting that stabilization of FOXP3 expression requires both mTOR and NFκB signal pathways. In vitro restimulation of TNFR2 agonist plus rapamycin-expanded Treg led neither to the loss of FOXP3 protein nor the enhancement of IL-17A production, especially under proinflammatory conditions, indicating a well-preserved Treg stability. TNFR2 knockout CD4+ T cells have increased expression of RORγt and IL-17 production, which is dependent on the impairment of TNFR2-mediated activation of NFκB [53]. We speculate that a similar process of regulation may exist in human Treg where TNFR2/NFκB signaling might act as a double-edged sword to enhance FOXP3 but also to inhibit RORγt expression, thus contributing to Treg stability. Another possible explanation is that TNFR2 engagement results in an autocrine TNF-TNFR2 loop, which further regulates the expression of histone methyltransferase EZH2 [51], a subunit of the polycomb repressor complex 2 (PRC2). EZH2 is known to bind to FOXP3 thus helping FOXP3 to regulate the gene transcriptional repression [54].

5. TNFR2 agonists and autoimmune diseases

Defect in the function of Treg as well as the low numbers are the main properties of various autoimmune diseases. Therefore, restoring the proper functional Treg

thus favoring the immune tolerance induction has become a final goal of treatment for patients with autoimmune diseases. As discussed above, ample studies show that either TNF and/or TNFR2 agonism has capacity to enhance Treg proliferation and activation. Furthermore, TNF-TNFR2 is essential to maintain the Treg function and stability in the inflammatory environment [44, 48]. Impaired TNF-TNFR signaling pathways occur in several human diseases including T1D, SLE, IBD, and MS. For instance, a single-nucleotide polymorphism (SNP) in the first intron is linked to a decreased level of TNFR2 in carriers of the SNP and a high risk of disease susceptibility [55]. T1D patients have higher $TNFR2^+$ Treg compared to healthy controls. The rationale for using TNFR2 agonists as a therapeutic option for autoimmune diseases was first shown in T1D. Using blood from patients with T1D, a dose-response relationship between TNFR2 agonism and the destroying of pathogenic autoreactive CD8 T cells was observed [56], suggesting inducing of TNF-TNFR2 pathway is an effective approach of selectively killing autoreactive T cells.

Currently used biologics targeting TNF include the anti-TNF antibodies infliximab, adalimumab, certolizumab, and the decoy receptor etanercept that binds to sTNF. Although they have a good safety profile, with increasing use of these drugs, paradoxical adverse events involving the skin, joints, and lungs have been described [57]. Skin manifestations are the most common adverse event and occur in about 25% of patients receiving anti-TNFs. The underlying mechanism is recently attributed to the TNFR2/A20 signal axis which is specifically responsible for TNF-mediated IL-17A inhibition [58]. Termination of NFκB activation is critical to prevent aberrant inflammatory responses. In memory CD4 T cells, A20 is identified as one of the strongest TNF-responsive genes with a strong inverse correlation to IL-17A expression.

6. TNFR2 antagonists and cancer immunotherapy

Tumor microenvironment preferably recruits TNFR2+ Treg cells which possess a highly immunosuppressive capacity, thus facilitating tumor immune escape. That TNFR2 knockout mice show improved immune responses to tumors might be caused by the lack of TNFR2 expressing Treg or have failed to develop systemic autoimmunity [59] or the decreased numbers and the impaired function of MDSCs [60]. In humans, the high level of TNFR2+ Treg is found in the peripheral blood of lung cancer patients [10] and in the tumor-associated ascites in ovarian cancer patients [61]. Moreover, the increased TNFR2 gene expression on Treg cells has been shown to be associated with exhaustion of CD8 cytotoxic T lymphocytes in metastatic melanoma patients.

In addition to being an inducer of Treg expansion, TNFR2 also acts as an oncogene which has been identified on at least 25 tumor types. Enhanced expression of TNFR2 on tumor itself has been also reported but not limited in human renal cell carcinoma, multiple myeloma, colon cancer, ovarian cancer, and cutaneous T-cell lymphomas (CTCL) [62]. In general, the overexpression of TNFR2 exploits this cytokine receptor for increased tumor cell proliferation and tumor growth. Genetic mutation/genomic gains of *TNFRSF1B*, a gene encoding TNFR2 protein, occur in patients with Sézary syndrome (SS), a rare form of CTCL often refractory to treatment. SS is characterized with high expression of TNFR2 on the tumor cells and Treg. Such gain-of-function mutation in TNFR2 leads to the enhanced noncanonical NKκB activation [63], a pathway primarily involved in cell expansion and growth. It seems being desirable to apply one approach that could successfully inhibit potent suppressive Treg and also directly prevent tumor growth by using the antagonistic molecules against TNFR2. Such TNFR2-specific blocking molecules would ideally inhibit Treg and permit Tconv proliferation and function, thus enabling to restore the antitumor immune responses and to induce tumor regression.

7. Strategies for blocking of TNF/TNFR2 signaling

A number of agonistic or antagonistic biological agents targeting to TNF and/or TNFR2 have been developed. Two potent dominant TNFR2 antagonist antibodies are developed by Faustman et al. group [64]. They report that these TNFR2 antagonists lock the TNFR2 receptor in the form of antiparallel dimmers, which further prevents the TNF binding as well as the intracellular scaffolding. Consequently, these dominant TNFR2 antagonists, even in the presence of TNF, could kill Treg isolated from ovarian cancer ascites more potently than it kills Treg from healthy donors. Interestingly, TNFR2 antagonistic mAbs are also able to directly kill TNFR2-expression ovarian cancer cell lines in vitro [64]. Similar effect is observed in another in vitro study where the cancer cells and lymphocytes were isolated from the end-stage SS patients [65]. In mouse model of colon and breast cancers, combining a blocking TNFR2 antibody with a kind of immune stimulant markedly enhances the antitumor efficacy of immunotherapy through reducing the number of tumor-infiltrating TNFR2+ Treg and increasing the number of IFNγ-producing CD8 cells [66].

Some pharmacological agents are found to regulate TNF and/or its receptors expression. Thalidomide and its analogues prevent the surface expression of TNFR2 on activated T cells, which is associated with the inhibition of TNFR2 protein trafficking to the cell membrane [67]. Treating acute myeloid leukemia patients with azacitidine and lenalidomide, a thalidomide derivative can reduce TNFR2 expression on T cells as well as TNFR2+ Treg in vivo, leading to enhanced effector immune function [68]. Cyclophosphamide is a DNA alkylating agent. It is commonly used as a cytotoxic chemotherapy in cancer treatment. In a mouse model, it is shown that cyclophosphamide treatment depletes TNFR2+ Treg via inducing the death of replicating Treg that co-express TNFR2 and KI-67 [69]. A re-expansion of Treg from lymphodepletion suppresses the effective antitumor immunity developed after cyclophosphamide treatment. Intriguingly, blockade of TNF signaling using etanercept inhibits TNFR2+ Treg cell expansion during recovery from cyclophosphamide-induced lymphodepletion and markedly inhibits the growth of established CT26 tumors in mice [70]. Altogether, it suggests that a TNFR2-targeted approach to inactive host Treg, especially in only tumor microenvironment, may offer optimal options for antitumor immune reactions.

8. Conclusions

Many surface receptors of Treg are also expressed on other immune cells, with TNFR2 being a prominent exception with highest density in the tumor microenvironment. TNFR2 is a functional receptor on Treg. Cell surface expression of TNFR2 not only identifies the potent Treg subsets but also is the property of tumor-infiltrating Treg. TNFR2 expression on some cancer-infiltrating Treg is about 100 times higher than on circulating Treg in control subjects. In other types of cancer, the abundance of TNFR2+ Treg in peripheral blood is higher than healthy ones. Targeting TNFR2 using small molecule agonists or antagonists is a promising but also a challenging task. Considering the suppressive property of Treg and its impaired functions in various immunopathologies, there is no doubt that novel (tumor-specific) antagonists against TNFR2 are promising for cancer immunotherapy. From the clinical utilities point of view, combination of TNFR2 inhibition with immune checkpoint inhibitors seems to be an attractive approach in reshaping modern cancer immunotherapy.

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Conflict of interest

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.

Nomenclature

IBD	Inflammatory bowel disease
CTCL	Cutaneous T-cell lymphomas
MS	Multiple sclerosis
MAPK	Mitogen-activated protein kinase
mTNF	Membrane-bound TNF
NFκB	Nuclear factor κB
RA	Rheumatoid arthritis
SNP	Single-nucleotide polymorphism
SS	Sézary syndrome
T1D	Type 1 diabetes
TACE	TNF-converting enzyme
TCR	T-cell receptor
TNFR	TNF receptor
TRAF	TNFR-associated factor
Treg	Regulatory T cells
TSDR	Treg-specific demethylated region

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References

[1] Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Science Translational Medicine. 2015;**7**(315):315ra189

[2] Haas J, Fritzsching B, Trubswetter P, Korporal M, Milkova L, Fritz B, et al. Prevalence of newly generated naive regulatory T cells (Treg) is critical for Treg suppressive function and determines Treg dysfunction in multiple sclerosis. Journal of Immunology. 2007;**179**(2):1322-1330

[3] Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nature Medicine. 2014;**20**(1):62-68

[4] Bovenschen HJ, van de Kerkhof PC, van Erp PE, Woestenenk R, Joosten I, Koenen HJ. Foxp3+ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. Joural of Investigative Dermatology. 2011;**131**(9):1853-1860

[5] Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, et al. Dysfunctional blood and target tissue CD4+CD25 high regulatory T cells in psoriasis: Mechanism underlying unrestrained pathogenic effector T cell proliferation. Journal of Immunology. 2005;**174**(1):164-173

[6] Keijsers RR, van der Velden HM, van Erp PE, de Boer-van Huizen RT, Joosten I, Koenen HJ, et al. Balance of Treg vs. T-helper cells in the transition from symptomless to lesional psoriatic skin. The British Journal of Dermatology. 2013;**168**(6):1294-1302

[7] Ohl K, Tenbrock K. Regulatory T cells in systemic lupus erythematosus. European Journal of Immunology. 2015;**45**(2):344-355

[8] Lyssuk EY, Torgashina AV, Soloviev SK, Nassonov EL, Bykovskaia SN. Reduced number and function of CD4+CD25 high FoxP3+ regulatory T cells in patients with systemic lupus erythematosus. Advances in Experimental Medicine and Biology. 2007;**601**:113-119

[9] Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nature Genetics. 2001;**27**(1):18-20

[10] Yan F, Du R, Wei F, Zhao H, Yu J, Wang C, et al. Expression of TNFR2 by regulatory T cells in peripheral blood is correlated with clinical pathology of lung cancer patients. Cancer Immunology, Immunotherapy: CII. 2015;**64**(11):1475-1485

[11] Moss ML, Jin SL, Milla ME, Bickett DM, Burkhart W, Carter HL, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. Nature. 1997;**385**(6618):733-736

[12] Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell. 1995;**83**(5):793-802

[13] Kim EY, Priatel JJ, Teh SJ, Teh HSTNF. Receptor type 2 (p75) functions as a costimulator for antigen-driven T cell responses in vivo. Journal of Immunology (Baltimore Md: 1950). 2006;**176**(2):1026-1035

[14] Chen X, Subleski JJ, Hamano R, Howard OM, Wiltrout RH, Oppenheim

JJ. Co-expression of TNFR2 and CD25 identifies more of the functional CD4+FOXP3+ regulatory T cells in human peripheral blood. European Journal of Immunology. 2010;**40**(4):1099-1106

[15] Chen X, Baumel M, Mannel DN, Howard OM, Oppenheim JJ. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. Journal of Immunology (Baltimore Md: 1950). 2007;**179**(1):154-161

[16] Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous clostridium species. Science. 2011;**331**(6015):337-341

[17] Annunziato F, Cosmi L, Liotta F, Lazzeri E, Manetti R, Vanini V, et al. Phenotype, localization, and mechanism of suppression of CD4(+) CD25(+) human thymocytes. The Journal of Experimental Medicine. 2002;**196**(3):379-387

[18] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: Mechanisms of differentiation and function. Annual Review of Immunology. 2012;**30**:531-564

[19] Hartigan-O'Connor DJ, Poon C, Sinclair E, McCune JM. Human CD4+ regulatory T cells express lower levels of the IL-7 receptor alpha chain (CD127), allowing consistent identification and sorting of live cells. Journal of Immunological Methods. 2007;**319**(1-2):41-52

[20] Koenen HJ, Joosten I. Antigen-specific regulatory T-cell subsets in transplantation tolerance regulatory T-cell subset quality reduces the need for quantity. Human Immunology. 2006;**67**(9):665-675

[21] Ermann J, Hoffmann P, Edinger M, Dutt S, Blankenberg FG, Higgins JP, et al. Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects from lethal acute GVHD. Blood. 2005;**105**(5):2220-2226

[22] Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;**322**(5899):271-275

[23] Ohta A, Sitkovsky M. Extracellular adenosine-mediated modulation of regulatory T cells. Frontiers in Immunology. 2014;**5**:304

[24] Himmel ME, MacDonald KG, Garcia RV, Steiner TS, Levings MK. Helios+ and Helios- cells coexist within the natural FOXP3+ T regulatory cell subset in humans. Journal of Immunology. 2013;**190**(5):2001-2008

[25] Bruder D, Probst-Kepper M, Westendorf AM, Geffers R, Beissert S, Loser K, et al. Neuropilin-1: A surface marker of regulatory T cells. European Journal of Immunology. 2004;**34**(3):623-630

[26] Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. Journal of Immunology. 2006;**176**(8):4622-4631

[27] Bin Dhuban K, d'Hennezel E, Nashi E, Bar-Or A, Rieder S, Shevach EM, et al. Coexpression of TIGIT and FCRL3 identifies Helios+ human memory regulatory T cells. Journal of Immunology. 2015;**194**(8):3687-3696

[28] Li X, Zheng Y. Regulatory T cell identity: Formation and maintenance. Trends in Immunology. 2015;**36**(6):344-353

[29] Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012;**482**(7385):395-399

[30] Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. Nature. 2013;**497**(7448):258-262

[31] Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. Journal of Immunology. 2010;**184**(7):3433-3441

[32] Akimova T, Beier UH, Wang L, Levine MH, Hancock WW. Helios expression is a marker of T cell activation and proliferation. PLoS One. 2011;**6**(8):e24226

[33] Gottschalk RA, Corse E, Allison JP. Expression of Helios in peripherally induced Foxp3+ regulatory T cells. Journal of Immunology. 2012;**188**(3):976-980

[34] Milpied P, Renand A, Bruneau J, Mendes-da-Cruz DA, Jacquelin S, Asnafi V, et al. Neuropilin-1 is not a marker of human Foxp3+ Treg. European Journal of Immunology. 2009;**39**(6):1466-1471

[35] Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proceedings of the National Academy of Sciences of the United States of America. 1975;**72**(9):3666-3670

[36] Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, et al. Human tumour necrosis factor: Precursor structure, expression and homology to lymphotoxin. Nature. 1984;**312**(5996):724-729

[37] Dempsey PW, Doyle SE, He JQ, Cheng G. The signaling adaptors and pathways activated by TNF superfamily. Cytokine & Growth Factor Reviews. 2003;**14**(3-4):193-209

[38] Rothe M, Pan MG, Henzel WJ, Ayres TM, Goeddel DV. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell. 1995;**83**(7):1243-1252

[39] Borghi A, Haegman M, Fischer R, Carpentier I, Bertrand MJM, Libert C, et al. The E3 ubiquitin ligases HOIP and cIAP1 are recruited to the TNFR2 signaling complex and mediate TNFR2-induced canonical NF-kappaB signaling. Biochemical Pharmacology. 2018;**153**:292-298

[40] Pan S, An P, Zhang R, He X, Yin G, Min W. Etk/Bmx as a tumor necrosis factor receptor type 2-specific kinase: Role in endothelial cell migration and angiogenesis. Molecular and Cellular Biology. 2002;**22**(21):7512-7523

[41] Catrysse L, Vereecke L, Beyaert R, van Loo G. A20 in inflammation and autoimmunity. Trends in Immunology. 2014;**35**(1):22-31

[42] Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, et al. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. Science (New York NY). 2000;**289**(5488):2350-2354

[43] Fischer JC, Otten V, Kober M, Drees C, Rosenbaum M, Schmickl M, et al. A20 restrains thymic regulatory T cell development. Journal of Immunology (Baltimore Md: 1950). 2017;**199**(7):2356-2365

[44] Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T. Cell phenotype in the inflammatory environment. Journal of Immunology (Baltimore Md: 1950). 2013;**190**(3):1076-1084

[45] Zaragoza B, Chen X, Oppenheim JJ, Baeyens A, Gregoire S, Chader D, et al. Suppressive activity of human regulatory T cells is maintained in the presence of TNF. Nature Medicine. 2016;**22**(1):16-17

[46] Okubo Y, Mera T, Wang L, Faustman DL. Homogeneous expansion of human T-regulatory cells via tumor necrosis factor receptor 2. Scientific Reports. 2013;**3**:3153

[47] Nie H, Zheng Y, Li R, Guo TB, He D, Fang L, et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-alpha in rheumatoid arthritis. Nature Medicine. 2013;**19**(3):322-328

[48] He X, Landman S, Bauland SC, van den Dolder J, Koenen HJ, Joosten I. A TNFR2-agonist facilitates high purity expansion of human low purity Treg cells. PLoS One. 2016;**11**(5):e0156311

[49] Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. Blood. 2006;**108**(1):253-261

[50] McGovern JL, Nguyen DX, Notley CA, Mauri C, Isenberg DA, Ehrenstein MR. Th17 cells are restrained by Treg cells via the inhibition of interleukin-6 in patients with rheumatoid arthritis responding to anti-tumor necrosis factor antibody therapy. Arthritis and Rheumatism. 2012;**64**(10):3129-3138

[51] Urbano PCM, Koenen H, Joosten I, He X. An autocrine TNFalpha-tumor necrosis factor receptor 2 loop promotes epigenetic effects inducing human Treg stability in vitro. Frontiers in Immunology. 2018;**9**:573

[52] Leclerc M, Naserian S, Pilon C, Thiolat A, Martin GH, Pouchy C, et al. Control of GVHD by regulatory T cells depends on TNF produced by T cells and TNFR2 expressed by regulatory T cells. Blood. 2016;**128**(12):1651-1659

[53] Miller PG, Bonn MB, McKarns SC. Transmembrane TNF-TNFR2 impairs Th17 differentiation by promoting Il2 expression. Journal of Immunology (Baltimore Md: 1950). 2015;**195**(6):2633-2647

[54] Arvey A, van der Veeken J, Samstein RM, Feng Y, Stamatoyannopoulos JA, Rudensky AY. Inflammation-induced repression of chromatin bound by the transcription factor Foxp3 in regulatory T cells. Nature Immunology. 2014;**15**(6):580-587

[55] Li D, Silverberg MS, Haritunians T, Dubinsky MC, Landers C, Stempak JM, et al. TNFRSF1B is associated with ANCA in IBD. Inflammatory Bowel Diseases. 2016;**22**(6):1346-1352

[56] Ban L, Zhang J, Wang L, Kuhtreiber W, Burger D, Faustman DL. Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(36):13644-13649

[57] Cleynen I, Vermeire S. Paradoxical inflammation induced by anti-TNF agents in patients with IBD. Nature Reviews Gastroenterology and Hepatology. 2012;**9**(9):496-503

[58] Urbano PCM, Aguirre-Gamboa R, Ashikov A, van Heeswijk B, Krippner-Heidenreich A, Tijssen H, et al. TNF-alpha-induced protein 3 (TNFAIP3)/A20 acts as a master switch in TNF-alpha blockade-driven IL-17A expression. The Journal of Allergy and Clinical Immunology. 2018;**142**(2):517-529

[59] Chen X, Hamano R, Subleski JJ, Hurwitz AA, Howard OM, Oppenheim JJ. Expression of costimulatory TNFR2

induces resistance of CD4+FoxP3- conventional T cells to suppression by CD4+FoxP3+ regulatory T cells. Journal of Immunology (Baltimore Md: 1950). 2010;**185**(1):174-182

[60] Polz J, Remke A, Weber S, Schmidt D, Weber-Steffens D, Pietryga-Krieger A, et al. Myeloid suppressor cells require membrane TNFR2 expression for suppressive activity. Immunity, Inflammation and Disease. 2014;**2**(2):121-130

[61] Govindaraj C, Scalzo-Inguanti K, Madondo M, Hallo J, Flanagan K, Quinn M, et al. Impaired Th1 immunity in ovarian cancer patients is mediated by TNFR2+ Tregs within the tumor microenvironment. Clinical Immunology. 2013;**149**(1):97-110

[62] Vanamee ES, Faustman DL. TNFR2: A novel target for cancer immunotherapy. Trends in Molecular Medicine. 2017;**23**(11):1037-1046

[63] Ungewickell A, Bhaduri A, Rios E, Reuter J, Lee CS, Mah A, et al. Genomic analysis of mycosis fungoides and Sezary syndrome identifies recurrent alterations in TNFR2. Nature Genetics. 2015;**47**(9):1056-1060

[64] Torrey H, Butterworth J, Mera T, Okubo Y, Wang L, Baum D, et al. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated Tregs. Science Signaling. 2017;**10**(462). pii: eaaf8608

[65] Torrey H, Khodadoust M, Tran L, Baum D, Defusco A, Kim YH, et al. Targeted killing of TNFR2-expressing tumor cells and Tregs by TNFR2 antagonistic antibodies in advanced Sezary syndrome. Leukemia. 2018 Oct 24. pii: 10.1038/s41375-018-0292-9

[66] Nie Y, He J, Shirota H, Trivett AL, Yang KDM, et al. Blockade of TNFR2 signaling enhances the immunotherapeutic effect of CpG ODN in a mouse model of colon cancer. Science Signaling. 2018;**11**(511). pii: 11/511/eaan0790

[67] Marriott JB, Clarke IA, Dredge K, Muller G, Stirling D, Dalgleish AG. Thalidomide and its analogues have distinct and opposing effects on TNF-alpha and TNFR2 during co-stimulation of both CD4(+) and CD8(+) T cells. Clinical and Experimental Immunology. 2002;**130**(1):75-84

[68] Govindaraj C, Madondo M, Kong YY, Tan P, Wei A, Plebanski M. Lenalidomide-based maintenance therapy reduces TNF receptor 2 on CD4 T cells and enhances immune effector function in acute myeloid leukemia patients. American Journal of Hematology. 2014;**89**(8):795-802

[69] van der Most RG, Currie AJ, Mahendran S, Prosser A, Darabi A, Robinson BW, et al. Tumor eradication after cyclophosphamide depends on concurrent depletion of regulatory T cells: A role for cycling TNFR2-expressing effector-suppressor T cells in limiting effective chemotherapy. Cancer Immunology, Immunotherapy: CII. 2009;**58**(8):1219-1228

[70] Chang LY, Lin YC, Chiang JM, Mahalingam J, Su SH, Huang CT, et al. Blockade of TNF-alpha signaling benefits cancer therapy by suppressing effector regulatory T cell expansion. Oncoimmunology. 2015;**4**(10):e1040215