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Contrasting effects of TNF and anti-TNF on the activation of effector T cells and regulatory T cells in autoimmunity

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Abstract

Anti-TNF treatment is effective in a majority of rheumatoid arthritis (RA), however, this treatment can unexpectedly trigger the onset or exacerbate multiple sclerosis (MS). Recent progress in cellular immunology research provides a new framework to analyze the possible mechanism underlying these puzzling contradictory effects. The delicate balance of protective CD4⁺FoxP3⁺ regulatory T cells (Tregs) and pathogenic CD4⁺FoxP3⁻ effector T cells (Teffs) is crucial for the outcome of anti-TNF treatment of autoimmune disease. There is convincing evidence that TNF, in addition to stimulating Teffs, is able to activate and expand Tregs through TNFR2, which is preferentially expressed by Tregs. Therefore, the contrasting effects of TNF on Tregs and Teffs are likely to determine the therapeutic effect of anti-TNF treatment. In this review, we discuss the current understanding of the general effect of TNF on the activation of T cells, and the impact of TNF on the function of Teffs and Tregs. Understanding the differential effects of TNF on Teffs and Tregs is fundamentally required for the design of more effective and safer anti-TNF or anti-TNF receptor(s) therapeutic strategy for autoimmune diseases.

Keywords

TNF; TNFR2; regulatory T cells (Tregs); effector T cells (Teffs)

1. Introduction

1.1. Contrasting immunopathogenic and immunoprotective effects of TNF

The pleiotropic cytokine tumor necrosis factor- α (TNF) is a major participant in the initiation and orchestration of complex events in inflammation and immunity [1]. Upon stimulation by pathogens and inflammatory signaling, TNF is produced primarily by immune cells, such as macrophages and T and B lymphocytes, as well as by other somatic cell types, including endothelial cells, mast cells, neuronal tissues and tumor cells [2-3]. Initially, transmembrane TNF is synthesized [4], and it can be subsequently released as

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soluble TNF upon cleavage by the metalloprotease TNF- α -converting enzyme (TACE or ADAM17) [5]. Both the soluble and transmembrane forms of TNF are biologically active in their trimeric forms [5].

The effects of TNF are mediated by two structurally related, but functionally distinct receptors, TNFR1 (or p55) and TNFR2 (or p75) [6]. These receptors also can be released from the cell surface as soluble forms by proteolysis and have the capacity to neutralize the action of TNF [7-8]. In contrast to the ubiquitous expression of TNFR1, TNFR2 is more restricted to lymphocytes and is more efficiently activated by transmembrane as opposed to soluble TNF [9]. TNFR1 with its death domain (DD) is the primary signaling receptor on most cell types and accounts for the majority of the proinflammatory, cytotoxic and apoptotic effects classically attributed to TNF [10-11]. In contrast, TNFR2 lacks an intracellular death domain and predominantly mediates signals promoting lymphocyte activation and proliferation [12-13].

TNF has well-documented proinflammatory effects. Nevertheless, increasing evidence reveals that TNF also has unexpected anti-inflammatory and immunosuppressive effect, especially after prolonged exposure [reviewed in ref.[14-16]]. Several transgenic mouse strains overproducing TNF consistently develop autoimmune disorders [reviewed in ref. [17]]. However, transgenic non-obese diabetic (NOD) mice over-expressing TNF in their pancreatic islets failed to develop autoimmune diabetes [18] and repeated injection of TNF paradoxically suppressed both type I diabetes in NOD mice and lupus nephritis in susceptible mouse strains [19]. Furthermore, NZB mice deficient in TNF unexpectedly exhibited acceleration of autoimmunity and lupus nephritis [20]. C57BL/6.129 mice deficient in TNF developed mild autoimmunity resembling the initial stages of lupus nephritis [21]. TNF knockout (KO) mice developed prolonged and exacerbated experimental autoimmune encephalomyelitis (EAE), although with a delayed onset, after EAE induction [22]. Thus, in mouse models of autoimmunity, TNF can either promote or inhibit inflammatory responses depending on complex factors which may include disease stage, background genetic susceptibility, length and timing of TNF expression.

Anti-TNF therapy is clearly beneficial for most of rheumatoid arthritis (RA) patients. Perhaps reflecting the strikingly contrasting activities of TNF, anti-TNF therapy in patients with RA and inflammatory bowel disease (IBD), however, a minority of patients develop lupus and neuroinflammatory diseases [21]. Furthermore, multiple sclerosis (MS) patients treated with anti-TNF agents resulted almost uniformly in immune activation and exacerbation of disease [21]. To date, the cellular and molecular mechanism underlying the contrasting proinflammatory and immunosuppressive effect of TNF, as well as anti-TNF agents, in autoimmunity remain to be clarified.

1.2. Immune equilibrium between regulatory T cells and effector T cells determines the outcome of autoimmune responses

Extensive studies provide compelling evidence that CD4⁺FoxP3⁺ regulatory T cells (Tregs) play an indispensable role in maintaining immune homeostasis and in suppressing deleterious excessive immune responses [23]. The induction of Treg suppressive activity is antigen specific and requires stimulation through the TCR, however, the suppressive function of Tregs is not antigen specific [24]. Therefore, a wide range of immune responses can be inhibited by Tregs through “bystander” suppression [25]. It is known that the cellular targets of Tregs include CD4 cells [26], CD8 cells [27], NK cells [28], NKT cells [29], DCs [30], macrophages [31] and B cells [32]. The *in vitro* suppressive activity of Tregs depends on cell-to-cell contact [26]. Several molecules, such as IL-10, TGF, CTLA-4, IDO and granzyme/perforin are reported to contribute to the suppressive activity of Tregs [reviewed

in ref. [33]]. However, the exact molecular mechanisms responsible for the cell contact dependent suppression by Tregs remain elusive.

Autoreactive effector T cells (Teffs) exist even in the healthy individual and their activation is persistently suppressed by Tregs, since removal of Tregs can result in the expansion and activation of these autoreactive Teffs [34]. Breakdown of tolerance by favoring the activities of Teffs over Tregs is a hallmark of autoimmune diseases [23]. The outcome of the autoimmune process is therefore largely determined by factors which tip the balance between the activation of Teffs and Tregs. The immune equilibrium between Teffs and Tregs, therefore, determines the cellular and molecular basis of immunopathological and immunoprotective effects of TNF. The capacity of TNF to expand the pool of functional Tregs represents the major negative feedback loop by which TNF counters its potent pro-inflammatory effects.

1.3. The complex relationship between protective Tregs and pathogenic Th17 cells

Upon activation by antigenic and cytokine stimulation, naïve CD4 cells can differentiate into different lineages of T helper subsets with a unique cytokine profile and effector functions [35]. Recent compelling evidence shows that IL-17-producing T lymphocytes comprise a distinct lineage of proinflammatory T helper cells, termed Th17 cells, that are major contributors to autoimmune disease [36-37]. Intriguingly, both Th17 cells and immunosuppressive Tregs derived from naïve CD4 cells can be reciprocally induced, contingent upon the presence of either IL-6 or IL-2, respectively, in the presence of TGF [37-39]. However, during the process of differentiation, both FoxP3 and ROR t, the transcription factors specific for Tregs and Th17 cells respectively, can occasionally be expressed by one cell at the same time [40]. Furthermore, it has been shown that Tregs exhibit “plasticity” and can become IL-17-producing cells after treatment with IL-6 both *in vitro* [41] and *in vivo* [42-43]. The functional relationship of Th17 and Tregs is also complicated. It has been clearly shown that both Th1 and Th2 responses can be potently suppressed by Tregs, however, Th17 responses are not always susceptible to Treg-mediated inhibition [44-46]. Actually, Tregs can promote *in vitro* generation of Th17 cells [39], as confirmed by our own experiments. This paradoxical effect of Tregs has pathophysiological relevance, since in a model of a T cell-mediated systemic autoimmune disorder resembling graft-versus-host disease, adoptive transfer of Tregs promoted the generation of Th17 cells [47]. Thus, the complex conversion to and support by Tregs of Th17 cells may also contribute to the puzzling effect of TNF and anti-TNF on the outcome of an autoimmune inflammation.

T cells play a critical role in the initiation and sustain an inflammatory response, nevertheless, many other types of cells are also involved in the pathogenesis of autoimmune disorders [48]. The direct and indirect responses of these non-T cells to anti-TNF, although representing major aspect of therapeutic effect, are beyond the scope of this review.

2. TNF activates Tregs through TNFR2

2.1. Human and mouse Tregs preferentially express high levels of TNFR2

We found that the majority (>80%) of thymic Tregs in normal mice express TNFR2 [49]. In normal mouse peripheral lymphoid tissues, TNFR2 is expressed primarily by CD4 T cells, in contrast, CD8 T cells express markedly lower levels of TNFR2 [49]. Compared with thymic Tregs, TNFR2-expressing Tregs are reduced to 30~40% in the peripheral lymphoid organs and further reduced to 10% in the circulation. Nevertheless, TNFR2 is still preferentially expressed by Tregs, since fewer than 10% of Teffs in the LNs and spleen of normal mice express lower levels of TNFR2 on a per cell basis [49-50].

TNF is expressed in human and mouse thymus and participates the development of thymocytes [51-53]. It is possible that either TNF or LT, which also uses TNFR2, actually contribute to the thymic differentiation and generation of Tregs. Similar to their mouse counterparts, all human thymic Tregs, but not conventional thymocytes, express TNFR2 [54]. Recently it has been shown that TNF-TNFR2 interaction plays a critical role in the expansion of human CD4⁺ and CD8⁺ Tregs [55-56]. Although TNF does not convert, but proliferatively expands FoxP3⁺ Tregs in the periphery. Human circulating FoxP3⁺ cells present in CD25^{hi}, CD25^{low} and even CD25⁻ subsets of CD4⁺ cells expressed markedly higher levels of TNFR2 (~70%), as compared with CD4⁺FoxP3⁻ T cells (~20%) [50,57-58]. TNFR2 is also expressed on antigen-specific CD4 Tregs induced by tolerogenic DCs [55] and CD8⁺ Tregs generated by anti-CD3 treatment [56]. The functional implications of TNFR2 in human Treg activity also are becoming evident. For example, both activated human and mouse Tregs produce high levels of soluble TNFR2 which contribute to their immunosuppressive activity [57]. TNF-TNFR2 interaction promotes the survival of Tregs in cancer and at inflammatory sites by inducing thioredoxin-1, a major antioxidative molecule, of Tregs in a NF B-dependent manner [59]. Furthermore, as in mice, TNF-TNFR2 interaction increases FoxP3 expression by human Tregs (our unpublished data).

2.2. Expression of TNFR2 is an indicator of maximal suppressive cells

Importantly, the expression of TNFR2 actually identifies the maximally suppressive subset of mouse Tregs [49-50]. For example, the majority of tumor infiltrating Tregs are highly suppressive TNFR2⁺ cells [49], and depletion of TNFR2⁺ Tregs results in tumor eradication after cyclophosphamide treatment [60]. TNFR2⁺ Tregs are highly proliferative in vivo presumably upon inflammatory stimulation by self antigens and TNF/LT [50]. Tregs deficient in TNFR2 are not able to control inflammatory responses in vivo [57]. In an ongoing inflammatory condition, up-regulation of TNFR2 expression on human Tregs also can serve as an indicator of more suppressive Tregs, as shown in malaria patients [61]. TNFR2 expression on human Tregs present in the synovial fluid of rheumatoid arthritis (RA) patients is also up-regulated [58], presumably reflecting their enhanced suppressive capacity [62]. It is not clear why TNFR2⁺FoxP3⁺ Tregs are more functionally suppressive.

2.3. TNF-TNFR2 interaction co-stimulates activation of T cells

It is well known that TNF has the capacity to promote TCR-dependent T cell activation [63], an effect attributed to the optimal activation of NF B by TNF [64]. Interaction of TNF with TNFR2, but not TNFR1, is directly co-stimulatory to TCR-mediated T cell activation, as demonstrated by the effects of agonistic monoclonal antibodies to TNFR2 on human CD4 cells [65]. TNFR2 KO mouse studies further confirmed the role of TNFR2 as a T cell co-stimulatory molecule. TNFR2 deficiency resulted in the reduced proliferative ability of CD4 cells as well as CD8 cells, and decreased production of IFN, TNF and IL-2 expression in response to TCR stimulation [66-68]. Remarkably, co-stimulations via TNFR2 and CD28 have different cellular effects and signaling requirements [65], and activation of CD28 is unable to rescue the defective response of TNFR2⁻ deficient T cells to TCR stimulation [23], indicative of an unique and non-redundant co-stimulatory role of TNFR2 on T cells. Furthermore, these studies also revealed that TNFR2 is important for the survival of T cells during a proliferative response and this was associated with up-regulation of anti-apoptotic molecules such as Bcl-xL, Bcl-2 and survivin [66-67]. T cell-derived TNF appears to be crucial for the co-stimulation of T cells [69]. Intriguingly, a recent study reports that TNF produced by the activated T cells is necessary for the stimulation of expansion and enhancement of suppressive function of Tregs [70]. This study in agreement with our previous observation that TNF, in addition to activating T cells, has the capacity to activate and expand Tregs [71].

2.4. Prolonged interaction of TNF-TNFR2 impairs TCR signaling

Although relatively brief exposure to TNF has a co-stimulatory effect on T cells, its role after prolonged exposure appears to be quite different. Chronic exposure to rTNF led to impaired production of cytokines such as IL-2, IFN, IL-4, IL-10, TNF and LT from both human T cells and T cells from TCR-tg mice [72-73] (reviewed in ref [15]). Attenuation of T cell activation was associated with defective Ca^{++} responses and could be partly attributed to decreased surface CD3 expression and attenuated LAT and PLC tyrosine phosphorylation [74] or down-regulation of CD28 expression [75], indicating that intact proximal TCR signaling can be disrupted by chronic TNFR signaling. This inhibitory effect of chronic TNF on human T cell activation was shown to be TNFR2-dependent [76]. In human CD4 and CD8 T cells, prolonged cross-linking TNFR2 had the capacity to down-regulate TCR/CD28-induced Ca^{++} mobilization, inhibited the expression of IL-2 mRNA and production of IL-2 and IL-10, indicating that persistent TNFR2 ligation can have negative effects on TCR-dependent signaling [65]. It is unclear whether chronic TNF stimulation or TNFR2 ligation attenuates TCR signaling in both Treg and Teff cells in the same manner. Since TNFR2 is predominantly expressed by humans and mouse Tregs [49-50,57-58], TCR signalling of Tregs is likely to be more profoundly affected by chronic TNF stimulation. It is possible that the previously observed down-regulation of TCR signalling in unfractionated T cells could result from the increased suppressive capacity of Tregs which can be activated and expanded by chronic TNF exposure. This scenario is supported by the accumulation of highly suppressive Tregs in the inflamed synovial fluid of RA patients [77]. Further study is warranted to elucidate these possibilities.

2.5. The role of TNFR1 in the activation of T cells

Although TNFR2 has been widely accepted as a co-stimulatory molecule of T cells, the ubiquitously expressed TNFR1 also contributes to T cell activation. Unlike other death domain receptors, TNFR1 is a TNF receptor-associated factor 2-binding receptor [78] that initiates cell activation, stimulate differentiation and promote survival. Based on studies of TNFR1 KO mice, TNFR1 was found to play a critical role in T cell responses to alloantigen [79] and autoantigen [80]. Recently, it was reported that TNFR1 can also act as a positive co-stimulatory molecule that is necessary for the initiation of T cell responses [81] and promotes T cell survival [82]. TNFR1 may mediate the suppressive effect of TNF on T cell activation. For example, TNFR1 was reported to mediate the effect of TNF on the impairment of proximal TCR signaling in mouse and Jurkat T cells [83]. We have shown that Treg-activating effect of TNF is mediated by TNFR2 [71], further study is needed to examine whether TNF-TNFR1 interaction would preferentially activate Teffs.

3. The responses of Teffs and Tregs to TNF and anti-TNF agents

3.1. TNF-TNFR2 interaction increases the resistance of Teffs to Treg-mediated inhibition

Our current understanding regarding the effects of TNF on the function of Teffs was mainly shaped by studies based on TNF KO and TNFR KO mice. For example, TNF KO mice are highly susceptible to challenge with an infectious agent (*Candida albicans*), and are resistant to the lethal dose of lipopolysaccharide (LPS) [84], suggesting critical role of TNF in T cell immunity. This view point has been verified by the observation that the risk of serious infections and malignancies was increased in patients with rheumatoid arthritis treated with anti-TNF antibody therapy [85]. The role of TNF in T cell immune responses has been extensively reviewed [86].

A recent study from our lab has provided new insight concerning the role of TNF-TNFR2 signaling in the activation of Teffs in the presence of persistent exposure of Tregs. We confirmed reports of other groups that exogenous TNF could promote proliferative

responses and cytokine production of Teffs to TCR stimulation [71]. Furthermore, Teffs proliferated despite the presence of Tregs during a brief period of stimulation with TNF, although more prolonged treatment with TNF restored the suppression by Tregs [71]. This effect of TNF on Teffs became more dramatic when it was combined with IL-1 and IL-6 [87]. TNFR2 is expressed at very low levels of <10% on resting Teffs, but can be quickly induced by TCR stimulation to ~30% even in the presence of Tregs [71]. TNFR2 is associated with greater suppressive functions when expressed by Treg cells, but is also associated with greater resistance to suppression when expressed by Teff cells. In mice bearing 4T1 breast tumor or Lewis Lung Carcinoma (LLC), intratumoral Teff cells expressing elevated levels of TNFR2 acquired the capacity to resist suppression by lymph node (LN)-derived Treg cells. However, they remained susceptible to inhibition by more suppressive tumor infiltrating Treg cells which expressed higher levels of TNFR2. Our data indicate that TNFR2 also co-stimulates Teff cells and induces their resistance to Tregs [50]. This is supported by a previous study showing that TNF-TNFR2 signals were critical for effective priming, proliferation, and recruitment of tumor-specific immunoreactive Teff cells [88].

3.2. Effect of TNF on differentiation of pathogenic Th17 cells

The effect of TNF on the Th17 responses could provide a key to demystify the puzzling action of TNF and anti-TNF treatment in autoimmunity. An initial report indicated that TNF had the capacity to amplify the generation of Th17 cells by IL-6 and TGF 1 [39]. However, this has not been confirmed by our own data and many other reports. Furthermore, neutralization of TNF with murine TNFR2-Fc fusion protein surprisingly exacerbated skin inflammation and markedly enhanced Th17 function and expression of the proinflammatory cytokines such as IL-1, IL-6, IL-17, IL-21, and IL-22, but suppressed FoxP3 expression in the skin and reduced the number of FoxP3-positive Treg cells in the draining LNs [89]. Thus, the surprising effect of TNF blockade on stimulating Th17 response and inhibiting Treg activity may account for the exacerbation of skin inflammation in some patients who receive anti-TNF treatments.

This has been confirmed in a mouse model of RA, in which TNF blockade using TNFR-Fc fusion protein or anti-TNF monoclonal antibody reduced the severity of arthritis but, unexpectedly, expanded the populations of Th1 and Th17 cells, which were shown by adoptive transfer to be pathogenic. Th1 and Th17 cell populations were also expanded in collagen-immunized TNFR p55(-/-) but not p75(-/-) mice. The expression of IL-12/IL-23 p40 was up-regulated in LN from p55(-/-) mice, and the expansion of Th1/Th17 cells was abrogated by blockade of p40. Treatment of macrophages with rTNF also inhibited p40 production in vitro. These findings indicate that at least one of the ways in which TNF-TNFR2 interactions regulate Th1/Th17 responses in arthritis is by down-regulating the expression of p40. Although TNF blockade increased the numbers of Th1 and Th17 cells in LN, it inhibited their accumulation in the joint, thereby providing an explanation for the paradox that anti-TNF therapy ameliorates arthritis despite increasing numbers of pathogenic T cells [90]. The results of this mouse model study have been confirmed by a human study. In RA patients, Th17 cells and IL-17 production increase after anti-TNF therapy, irrespective of disease activity. CCR6 expression is up-regulated on Th17 cells, but RA patients in remission on anti-TNF therapy have significantly lower expression than those with active disease. The increase in peripheral Th17 cells in RA patients after anti-TNF therapy is accompanied by a decrease in Th17-specific CCR6 expression, which might prevent homing of these potentially pro-inflammatory cells to the synovium [91].

Taken together, this data suggest that TNF may suppress Th17 responses, while promoting Treg activity. Blockade of TNF thus has the potential to promote Th17 responses at the expense of Treg activity, which together exaggerate autoimmune inflammation. In contrast,

with the effects of anti-TNF in RA, suppression of TNF has no effects on SLE and even exacerbates MS. It would be simplest to propose this to be due to greater interference with Tregs rather than with Teffs, but this remains to be clearly established since other factors such as impaired penetration of the blood-brain barrier and a role for non-TNF dependent inflammatory mediators may be contributing.

3.3. Interaction of TNF and TNFR2 is crucial for the generation and suppressive function of human Tregs

We and others have shown that, as in mice, human Tregs also express characteristically high levels of TNFR2 [50,54,57-58]. Thus, we favor the idea of Bilate [92] that, as an evolutionarily conserved innate and adaptive cytokine, TNF should similarly activate human Tregs as well. Indeed, our own preliminary data suggest that TNF could activate normal human Tregs (data not shown). More recent studies discussed in the following sections also lend support to this notion.

For example, vitamin D3-treated human dendritic cells (VD3-DCs) are able to induce human CD4⁺FoxP3⁺ Tregs that suppress proliferation of responder T cells in vitro [93]. One characteristic of such tolerogenic VD3-DCs is their unique capacity to produce large amounts of TNF [94], and to express markedly higher levels of membrane-bound TNF (mTNF) than untreated DCs [55]. Importantly, inhibition of TNF with anti-human TNF Ab (adalimumab, blocking both sTNF and mTNF) during induction of Tregs by VD3-DCs abrogates the suppressive function of Tregs. Furthermore, blocking TNFR2 by anti-human CD120b Ab (hTNFR-M1) during Treg induction similarly abrogated their subsequent suppressive function. In contrast, etanercept, a TNFR2-Fc fusion protein which mainly blocks soluble TNF (sTNF), did not change the suppressive capacity of Tregs. This study thus indicates that interaction of mTNF expressed on tolerogenic DCs with TNFR2 expressed on Tregs is crucial for the induction of suppressive function of human CD4 Tregs [55]. In another example, anti-CD3 mAb OKT3 can also induce FoxP3 expression and suppressive function of human CD4 cells [95], it would be interesting to ask whether TNF plays a role or not, since it was reported that the induction of CD8⁺ Tregs by anti-CD3 mAb was also TNF-dependent [56].

3.4. TNF promotes the survival and function of Tregs in the inflammatory environment

Accumulation of Tregs in different cancers and inflammatory diseases may result from aberrant proliferation as well as greater resistance to oxidative stress compared with conventional T cells. It was shown that freshly isolated Tregs are resistant to oxidative stress-induced cell death compared with Teff cells and maintain their suppressive function, even at H₂O₂ dosages lethal for Teffs [96]. Human Tregs express and secrete higher levels of thioredoxin-1 (Trx-1), a major antioxidative molecule. Trx-1 has an essential role in maintaining their surface thiol density as the first line of antioxidative defense mechanisms [59]. Inflammatory stimuli led to an up-regulation of Trx-1 expression on Tregs, but only TNF increased Trx-1 release. In conjunction with increased expression and secretion of Trx-1 on Tregs but not on Teffs, TNF also enhanced surface thiol expression and consequently increased their resistance to H₂O₂. This effect of TNF on Tregs was NFκB-dependent, since inhibition of NFκB abolished the TNF-induced increase of surface thiols as well as Trx-1 secretion [59]. Similar to our observation of collaborative effects of IL-2 and TNF on the up-regulation of FoxP3 expression and suppressive function of mouse Tregs, IL-2 also synergized with TNF in the induction of Trx-1 release [59]. In agreement with the conclusion drawn from our mouse Treg study, this study clearly indicates that TNF represents a dynamic anti-inflammatory feedback mechanism that promotes Treg activity within inflammatory milieus to prevent prolonged or excessive immune responses.

3.5. In vitro effect of TNF on the function of human Tregs

Since TNF clearly stimulates TNFR2⁺ Tregs, what accounts for the therapeutically beneficial effects of anti TNF in RA? Ehrenstein and colleagues studied the effect of exogenous TNF (0.1~5 ng/ml) on co-cultures of CD4⁺CD25^{hi} cells and CD4⁺CD25[−] cells from normal human healthy donors or from RA patients responsive to anti-TNF therapy. Their results showed that exogenous TNF neither increased nor decreased PB Treg activity, as evidenced by no alteration of percentage inhibition of proliferation in the co-cultures [97]. Another study also did not detect any impact of exogenous TNF on Tregs [98]. Valencia and colleagues examined the effect of higher concentrations of TNF (50 ng/ml) on in vitro normal human Treg activity. They reported that exogenous TNF down-regulated FoxP3 expression and blocked Treg suppressive activity by signaling through TNFR2 and that an agonist monoclonal antibody to TNFR2 also reversed the suppressive activity of healthy donor PB CD4⁺CD25^{hi} cells [99]. In agreement with the results of this study, Nagar and colleagues recently reported that although FoxP3 expressed by Tregs was not down-regulated by TNF, TNF decreased the suppressive activity of human peripheral blood (PB) CD45RA[−] Tregs, based on their evidence that TNF stimulated the proliferation and cytokine production in co-cultures of Tregs and Teffs [58]. However, rather than decreasing Treg activity, their results may be attributable to the capacity of TNF to enhance the response of Teffs to TCR stimulation. Nagar et al's report showing that the levels of TNF-induced INF γ in their Treg-Teff co-cultures actually paralleled the levels in unstimulated co-cultures [58], indicating that the degree of suppression by Tregs was not really diminished by TNF. Consequently, the beneficial effect of anti-TNF therapy may partly result from restoring the equilibrium between Teffs and Tregs by suppressing the activation of Teffs and sensitizing Teffs to Treg-mediated inhibition.

3.6. Effects of anti-TNF treatment on the function of Tregs

TNF is generally considered to have major proinflammatory effects in autoimmune diseases based on studies of the anti-inflammatory effects of anti-TNF. Several such studies also show that both expression levels of FoxP3 and suppressive function of CD4⁺CD25^{hi} cells are increased after neutralization of TNF [97,99]. Since anti-TNF treatment effectively suppresses autoimmune responses and Tregs are potent suppressors of autoreactive cells, it is therefore logical to predict that anti-TNF should restore or improve Treg activity [97,99]. However, these clinical results need to be interpreted more carefully, simply because the proportion of activated CD25^{hi} Teffs contaminating the "CD4⁺CD25^{hi} Treg" population could also be reduced by anti-TNF treatment. The studies claim that anti-TNF treatment reduce apoptosis of Tregs in RA [100] may neglect the possibility that the replication of Tregs in an inflamed site may also be reduced by neutralization of TNF [48,101]. Furthermore, "Tregs" reportedly reduced by anti-TNF treatment in autoimmune patients or animal models, are actually not *bona fide* naturally occurring CD4⁺FoxP3⁺ Tregs [97,102-103], but are likely to be IL-10-producing Tr1 or TGF β -expressing Th3 cells (please see our review in [77] for detailed discussion).

Recently, accumulating evidence points in the opposite direction, that anti-TNF at times eliminates Treg activity. For example, in the mouse psoriasis model, anti TNF therapy suppressed FoxP3 expression in the skin and the number of FoxP3-positive Treg cells was reduced in the draining LNs [89]. The stimulatory effect of pathogenic Teffs on Tregs could also be attenuated by neutralization of TNF [104]. Induction of suppressive function of Ag-specific human CD4⁺ Tregs by vitamin D3-treated tolerogenic DCs [55] or human CD8⁺ Tregs by anti-CD3 mAb were blocked by anti-TNF or anti-TNFR2 Abs [56]. Further studies are warranted to determine whether impaired Treg activity is responsible for the non-responsiveness or detrimental effect of anti-TNF agents in some autoimmune disorders.

One approach to avoid potential impairment of Treg activity by neutralizing TNF is to selectively block interaction of TNF with TNFR1, the primary signaling receptor that initiates the majority of inflammatory responses classically attributed to TNF. A recent study showed that treatment with a TNFR1 inhibitor dramatically inhibited the development of mouse EAE and suppressed Th1 and Th17 responses as well as infiltration of inflammatory cells into the CNS [105]. Presumably, blockade of TNF-TNFR1 interaction will still allow the activation of Tregs through the TNF-TNFR2 pathway, and the activated Tregs are able to consequently contribute to the inhibition of autoimmune responses.

4. Concluding remarks

TNF can co-stimulate both Tregs and Teffs and this effect is likely to be mediated by the same receptor, TNFR2. In the resting state, TNFR2 is constitutively expressed by mouse and human Tregs, while markedly lower proportion of Teffs express low levels of TNFR2. Upon TCR stimulation, expression of TNFR2 is also up-regulated on Teffs, but still at markedly lower levels than TNFR2 expression on activated Tregs [49,71,106], suggesting that TNF-TNFR2 co-stimulatory pathway may preferentially activate Tregs. We favor a biphasic model: at the initial stage of host immune responses, TNF co-stimulates the activation of Teffs and liberates them from the suppressive effects of Tregs, and enables them to mount an effective immune responses against pathogens; at the later stage, higher levels of TNFR2 expression by Tregs enables them to outcompete Teffs for TNF. TNF-TNFR2 co-stimulated activation of Tregs may therefore account for the accumulation of activated Tregs found in more prolonged inflammatory responses as in autoimmunity, sepsis, some infection and tumors, and may also account for immunosuppression seen in the chronic exposure to TNF. The stimulatory effect of TNF on Tregs thus represents an important anti-inflammatory feedback mechanism responsible for the attenuation and termination of prolonged or excessive immune responses, which otherwise may cause severe collateral damage (Fig 1).

The biphasic effect of TNF on Teffs and Tregs may underlie both beneficial and detrimental effects of anti-TNF treatment in autoimmunity, by following three means. First, since TNFR2 is constitutively expressed on the most suppressive Tregs, while its expression on Teffs need to be induced and can be down-regulated by neutralization of TNF (our unpublished observation), it is possible that the beneficial effect of anti-TNF treatment is achieved in part by suppressing TNFR2 expression on Teffs and consequently sensitizing Teffs to Treg-mediated inhibition. Second, since providing TNF is critically required for the activation and proliferative expansion of Tregs, blockade of TNF may result in the down-regulation of Treg activity which may account for the detrimental effect of anti-TNF treatment in some autoimmune diseases such as MS. TNF-derived from activated pathogenic Teffs is critical for activation of Tregs [70]. This raises the third possibility that anti-TNF treatment may inhibit TNF-producing capacity of Teffs and indirectly blocks the stimulatory pathway of Teffs to Tregs. Despite the mechanistic basis for its beneficial effects, anti-TNF is not effective in SLE and exacerbates MS. Whether the equilibrium favors Teffs over Tregs in these conditions has not been clarified. The factors determining the outcome of anti-TNF treatment is likely to be complicated which may include timing, location, cytokine milieu, receptor usage, form (free vs membrane bound) and cellular source of TNF. Given the biphasic effect of TNF on Teffs and Tregs is mediated by TNFR2, the expression levels of this molecule on Tregs and Teffs is a key variable. Anti-TNF is also likely to have different effect in Th1-dominated or Th17-dominated autoimmune disorders, since the responses of Th1 cells and Th17 cells to Tregs and anti-TNF are distinct. Many variables concerning TNF-TNFR2 interactions need to be clarified to optimize anti-TNF therapeutic strategy, e.g., Th1 versus Th17 bias, the levels of TNFR2 on Teffs and Tregs and levels of soluble TNFR2 at inflammatory sites. Furthermore, the possibility that TNF-TNFR1 interaction preferentially activates Teffs should also be further studied.

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List of abbreviations

DC	dendritic cell
EAE	experimental autoimmune encephalomyelitis
LLC	Lewis Lung Carcinoma
MS	multiple sclerosis
mTNF	membrane-bound TNF
NOD	Non-Obese Diabetic
PB	peripheral blood
RA	rheumatoid arthritis
sTNF	soluble TNF
Teffs	effector T cells
Tregs	regulatory T cells
Trx-1	thioredoxin-1
VD3	vitamin D3

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Fig 1.

Natural and therapeutic resolution of inflammation by Tregs and anti-TNF. In the steady state, the equilibrium of the activation of Tregs and Teffs is crucial for immune homeostasis (1). In the early stage of inflammation, activated Teffs up-regulate their TNFR2 expression and increase their capacity to resist Treg-mediated inhibition, and therefore mount an effective immune response. In the later stage of inflammation, Tregs outcompete with Teffs for co-stimulatory TNF-TNFR2 action and enhanced their suppressive activity, resulting in the resolution of inflammatory responses and restoration of immune homeostasis (1). However, certain types of Teff cells (such as Th17 cells) may not be suppressed by Tregs and this causes persistent and excessive inflammation (3). Anti-TNF directly inhibits activation of Teffs and sensitizes them to Treg-mediated inhibition. Thus, intervention by anti-TNF can resolve inflammation and restore the immune homeostasis (1). Under some circumstances, such as sepsis, Tregs activated and expanded by inflammatory stimulation can suppress Teffs and transiently cause immunosuppression (4).