T Cells as Sources and Targets of TNF: Implications for Immunity and Autoimmunity

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Abstract

TNF is a pleiotropic cytokine produced by many cell types upon different stimuli and in various physiological and pathological conditions. In this review, we focus on the role of TNF in T cell responses as demonstrated by in vitro and in vivo observations in mice and humans. TNF has an impact on all aspects of T cell biology such as development in the thymus, peripheral homeostasis, primary antigenic responses, apoptosis, effector functions, memory cell formation and tolerance induction and maintenance. In most cases, TNF has an immunostimulatory role in T cell responses; however, under certain conditions, TNF can exert immunomodulatory effects on T cells. We also review how T cell-derived TNF is an important component of T cell immunity as exemplified by many studies involving intracellular pathogens and tumors. Finally, we summarize how TNF T cells interplay contributes to pathology in autoimmune disorders and what is known about the effect of widely used TNF blockers on T cell differentiation/function.

Thymus and TNF

There is plenty of evidence that TNF is expressed in the developing thymus of mice [1, 2] as well as in humans [3]. TNF is synthesized also in thymocytes in adult mice [4] and its mRNA is localized in the subcortical regions of medulla, a region related to thymocyte selection processes [5].

But what can be the role of TNF in the fetal or adult thymus? Most studies point towards a role for TNF in regulating lineage commitment and early thymocyte development by promoting both differentiation/proliferation (most likely by membrane-bound TNF, mTNF) and apoptosis of immature thymocytes [5, 6]. TNF is also shown to augment human T cell lymphopoiesis in irradiated NOD/SCID mice [7]. Altogether, it is proposed that TNF in the thymus plays an important role in
thymocyte production by delivering both positive and negative signals at early stages of differentiation.

Overproduction of TNF after lipopolysaccharide (LPS) injection results in apoptosis of double-positive thymocytes, which is completely abrogated when a-TNF Ab is coadministered [8], suggestive for a strong effect of TNF in thymocyte homeostasis under stressful conditions. The role of TNF in negative selection is less clear. Although a possible role in promoting negative selection was suggested by experiments with TNFRKO mice [9] or with fetal thymic organ cultures [10], other studies using class I and class II restricted TCR-transgenic (TCR-tg) mice failed to demonstrate a direct role of TNF in mediating thymocyte negative selection [11, 12]. It is probable that systems used do not really reflect physiological negative selection events and double-positive cell death observed can be due to cytokines or steroids resulting from activated T cells in the periphery. It is also possible that multiple coreceptors are cooperatively involved in negative selection [10, 13] and blocking one of them could lead to subtle defects only. TNF function in thymus can affect T cell tolerance not only by modulating negative selection but also by regulating Treg production [14], a topic reviewed elsewhere in this volume.

Role of TNF in T Cell Responses

Initial evidence that TNF plays a role in T cell responses came from studies on normal human T lymphocytes. Recombinant human TNF was demonstrated to enhance T cell proliferation in response to a variety of stimuli such as IL-2 [15], α-CD3 [16], alloantigen [17], or phorbol esters [18]. Moreover, rTNF promoted upregulation of MHC molecules [15], IFN-γ production [15, 19], expression of high-affinity IL-2R [18] and TNFR2 [19]. These effects can be well attributed to optimal NF-κB activation by TNF [18, 20].

Studies in human T cells highlighted a role for TNFR2, but not TNFR1, in delivering costimulatory signals distinct of CD28 [19]. The role of TNFR2 as T cell costimulatory molecule was confirmed in studies using TNFR2KO mice. TNFR2 ablation was shown to decrease proliferative capacity of CD4 and CD8 T cells, and reduces IFN-γ, TNF and IL-2 expression in response to α-CD3 crosslinking or antigenic stimulation [21, 22]. TNFR2 signaling was found to modulate AKT activity, as well as NF-κB activation. Remarkably, CD28 coligation was unable to rescue either defect [23]. In these studies, it was also shown that TNFR2 is important for the survival of T cells during the proliferative response and this was associated with upregulation of Bcl-xL, Bcl-2 and survivin expression [21, 22].

Studies from our lab extended the role of TNF in enhancing TCR-generated signals. TNFKO, polyclonal or TCR-tg, CD8 T cells showed an abnormally high TCR signal threshold for T cell responses, and this defect was restored by TNF provided from activated wild-type (WT) cells as shown in coculture experiments [24]. Apart
from the role of TNF as a costimulatory ligand in responses of naïve T cells to antigen, we showed that TNF has a serious impact on T cell tolerance, since TNF−/− T cells exhibited defective anergy induction and clonal deletion, as a result of altered signaling thresholds in response to self-antigen recognition. Additionally, we revealed a previously unrecognized role of endogenous TNF in the homeostasis of naïve CD8 T cells since TCR-tg TNF−/− naïve CD8 T cells had a survival defect when compared to their TNF+/+ counterparts. Moreover, naïve TCR-tg or polyclonal CD8 TNF−/− T cells, when transferred to lymphopenic recipients, undergo impaired homeostatic expansion, a process that induces TNFR2 expression on T cells [24]. In line with the role of endogenous TNF to activate T cells is the observation that B6.gld/gldTNF−/− mice exhibited an attenuated course of the generalized lymphoproliferative disorder and that was correlated with decreased peripheral T cell activation and lower concentration of IFN-γ in the serum [25].

Although relatively brief exposure to TNF has a costimulatory effect on T cells, its role after prolonged exposure appears quite different. Chronic exposure to rTNF led to impaired production of cytokines such as IL-2, IFN-γ, IL-4, IL-10, TNF and LT from both human T cells and T cells from TCR-tg mice [26, 27]. Attenuation of T cell activation was associated with defective Ca2+ responses and could be partly attributed to decreased surface CD3ζ expression and attenuated LAT and PLCγ tyrosine phosphorylation [28] or downregulation of CD28 expression [29], 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 [30], whereas in mouse and Jurkat T cells TNFR1 was found to be responsible for impairment of proximal TCR signaling [31].

The role of TNF/TNFR pathway in T cell death was first demonstrated by induction of apoptotic death of human and mouse T cell blasts in a Fas-independent manner [32]. In line with this finding, rTNF could enhance activation-induced death in memory and naïve T cells from aged humans [33, 34].

However, in two studies using TCR-tgTNFKO mice infected with LCMV, no apparent role of TNF in mediating CD8 T cell death was observed [35, 36]. Accordingly, in vivo neutralization of TNF had no impact on CD4 T cell apoptosis unless Fas pathway was defective, too [37]. Nevertheless, in vivo neutralization of TNF in another TCR-tg experimental model showed that TNF is responsible for controlling intrahepatic apoptosis of activated T cells and thus regulating peripheral T cell numbers [38].

Both receptors can mediate the cytotoxic effect of TNF on activated T cells as shown by early studies using agonistic a-TNFR Abs [33]. The role of TNFRs was directly assessed by studies using TNFRKO mice. Experiments with TNFR1KO- and TNFR2KO-activated T cells revealed that TNF mediates activation-induced cell death of CD8 T cells, but not CD4, through TNFR2 signaling [39, 40]. On the other hand, in vitro experiments with TNFR2KO CD8 T cells [23] showed that TNFR2 actually delivers survival signals during TCR stimulation probably through upregulation of Bcl-xL and activation of NF-κB [23], a factor that has been shown to protect
from TNF-induced cell death [41]. These data were also confirmed in vivo after engineered-LM infection of TCR-tg TNFR2KO mice, where increased CD8 T cell apoptosis was correlated with diminished expression of Bcl-2 and survivin [21].

Two rather similar studies involving infection of TCR-tg TNFR1KO mice with LCMV, revealed no role of TNFR1 in activated CD8 T cell death [35, 36]; however, it was shown that Fas and TNFR1 synergize to promote peptide-induced T cell deletion under limited conditions [36]. Other in vivo studies with TCR-tg TNFR1KO mice revealed a role of TNFR1 in activation-induced cell death of CD8 T cells only after challenge with low antigen concentrations, whereas in high concentrations both TNFR1KO and WT CD8 T cells declined with similar kinetics [42].

Studies in humans revealed that under certain pathological conditions T cells can be rather susceptible to TNF-induced cell death. In one study, T cells only from HIV-infected patients were sensitive to either a-TNFR1- or a-TNFR2-mediated apoptosis, but this was not correlated with differences in TNFR expression. Notably, susceptibility to TNFR-mediated death was associated with disease progress and was significantly decreased in patients treated with antiretroviral agents. What made T cells from HIV-infected donors poised for death was the lack of protection due to decreased levels of Bcl-2 and at the same time expression of active caspases 3 and 8 [43]. In another study, TNF- or TNFR2-agonistic antibodies could selectively kill activated CD8 T lymphocytes from type I diabetes (T1D) patients. It is of exceptional interest that only antigen-specific autoreactive CD8 T cells, but not other activated T cells, were susceptible to TNFR2-mediated apoptosis [44].

Taken together, the TNF/TNFR pathway(s) has definitely a role in apoptotic death of T lymphocytes (mostly CD8). However, the role of TNF is manifested mainly in conditions of low antigenic stimulation, whereas in T cells activated by high antigen doses and/or fully mature dendritic cells (DCs) more pathways are involved and may compensate for the lack of intact TNFR pathway(s).

Not only are there conditions where TNF is dispensable for T cell activation-induced cell death, but TNF can actually inhibit it. It is reported that a membrane form of TNF found on exosomes produced by synovial fibroblasts from rheumatoid arthritis (RA) patients can delay T cell death and sustain primary CD4 T cell proliferation after a-CD3 stimulation [45].

**TNF and T Cells in Immunity**

Early experiments in TNFKO or TNFRKO mice demonstrated the role of TNF in immune responses as manifested by reduced LPS-induced lethality, or by defective responses against a variety of pathogens such as *Corynebacterium parvum*, increased susceptibility to *Candida albicans* and *Listeria monocytogenes* [46–49] and inability to control infections with *Mycobacterium tuberculosis*, *M. avium* and *M. bovis* [50–52]. Moreover, with the development and widespread use of TNF blockers for treatment
of RA, Crohn’s disease, psoriasis and ankylosing spondylitis, it became evident that neutralization of TNF in humans can lead to reactivation of latent tuberculosis and development of lymphomas (both rare but life-threatening side effects) [53]. Since TNF is produced by many cell types including activated macrophages, activated CD4 and CD8 T cells, NK cells, DCs and other immune and nonimmune cells [54], it is conceivable that its production by a specific cell type has distinct biological consequences. It has been shown that naïve T cells from both humans and mice transcribe TNF mRNA early after initial TCR engagement, and especially in the case of CD8 T cells TNF protein can be detected (either membrane-bound or soluble) as early as 5 h after stimulation [55 and pers. obs.]. Proinflammatory and cytotoxic properties of T cell-derived TNF can have a dramatic impact on different cell types of the host but also can affect differentiation and/or promote apoptosis of T cells themselves. The role of T cell-derived TNF in protection against *M. tuberculosis* infection was assessed by Saunders et al. [56] by transferring T cells from WT or TNFKO mice to infected Rag1KO hosts. These experiments showed that only transfer of T cells competent for producing TNF could increase survival of infected mice probably through granuloma formation. However, T cell-derived TNF was not enough to control bacterial growth in most tissues, and mice finally succumbed, albeit at later time points. A later study from the same group [57] using mice expressing only mTNF showed that transmembrane TNF contributes to T cell migration and subsequent initial granuloma formation; transfer of mTNF-expressing T cells to Rag1KO or TNFKO mice was sufficient to control the acute phase of *M. tuberculosis* infection and prolonged survival of host mice. Accordingly, another study demonstrated that mTNF on memory CD8 T cells enhanced greatly (and to a lesser extent for CD4 T cells) their ability to control *Francisella tularensis* live vaccine strain intramacrophage growth in vitro [58]. Experiments with Armstrong LCMV-infected WT or TNFKO mice hosting P14/WT or P14/TNFKO transferred CD8 T cells indicated that TNF is not critical for the primary LCMV immune response [59]. However, at later time points, LCMV-specific T cell numbers were significantly higher in TNFKO hosts. This is consistent with observations in experiments using LCMV-infected TNFKO, TNFRDKO, or WT mice and measuring LCMV-specific CD4 T cells at relatively late time points [60]. However, the authors showed that this was due to an indirect role of TNF on T cells, since T cell-derived TNF can induce apoptosis of nonplasmacytoid DCs and in that way it can compromise T cell activation and proliferation. A consequence in LCMV-infected TNFRDKO mice was increased number of memory CD4 T cells, implying that TNF has a suppressive role in the protective responses of CD4 T cells against viruses [60]. Similar role for TNF in CD8 T cell memory cells was reported by the same group, but in this case TNF was directly regulating effector CD8 T cell apoptosis [61]. In another study using adenovirus infected TNFKO, TNFR1KO and TNFR2KO mice, it was demonstrated that TNF-TNFR interactions are required for optimal generation of CD8 T cell effector functions which result in optimal clearance of virally infected hepatocytes [62].