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TNF activity and T cells

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ABSTRACT

TNF (tumor necrosis factor) is both a pro-inflammatory and anti-inflammatory cytokine that is central to the development of autoimmune disease, cancer, and protection against infectious pathogens. As well as a myriad other activities, TNF can be a product of T cells and can act on T cells. Here we review old and new data on the importance of TNF produced by T cells and how TNF signaling via TNFR2 may directly impact alternate aspects of T cell biology. TNF can promote the activation and proliferation of naïve and effector T cells, but also can induce apoptosis of highly activated effector T cells, further determining the size of the pathogenic or protective conventional T cell pool. Moreover, TNF can have divergent effects on regulatory T cells. It can both downregulate their suppressive capacity, but also contribute in other instances to their development or accumulation. Biologics that block TNF or stimulate TNFR2 therefore have the potential to strongly modulate the balance between effector T cells and Treg cells which could impact disease in both positive and negative manners.

1. Introduction

Tumor necrosis factor (TNF, previously referred to as TNF-α) is a pleiotropic cytokine involved in the pathogenesis of a range of physiological processes that control inflammation, anti-tumor responses, and immune system homeostasis [1,2]. TNF is best known for its protective activity against pathogens, being a product of effector CD4 and CD8 T cells or innate cells, that can lead to killing of infected cells. It is also known for its activity in tissues, highlighted by the successes of the TNF inhibitors in patients with RA, psoriatic arthritis, Crohn's disease and other indications, where it mediates inflammatory activity, at least in part via effects in structural cells such as fibroblasts, endothelial cells, and epithelial cells. These actions are largely mediated by TNF receptor 1 (TNFR1, p55) [3–6] that is ubiquitously expressed by almost all cell types and has been suggested to be the primary receptor for soluble TNF. What has received less attention are the effects of TNF on T cells, even though the initial observations of a TNF receptor being induced in T cells or thymocytes after activation, and TNF controlling some functional responses, were reported many years ago [7–10]. In this article, we review both older and recent literature on the importance of TNF made by T cells, and the role of TNF in modulating T cell activity, which may primarily reflect the activity of TNFR2 (p75/p80) that can respond strongly to membrane-bound TNF as well as soluble TNF [11].

2. Role of TNF during T cell-mediated immunity or autoimmunity

Early studies in TNF-/- or TNFR-/- mice suggested both beneficial and harmful effects of TNF in immune responses [12–14], although the source of TNF for these activities was not clear. More recent studies with conditional ablation of TNF in T cells versus myeloid cells have clarified the role of T cell-derived TNF to a certain extent [15]. For example, TNF-/- or TNFR1-/- mice are resistant to lethal doses of lipopolysaccharide (LPS) or *S. aureus* enterotoxin B (SEB) but are severely impaired in clearing *Listeria monocytogenes*. Specific TNF ablation in T lymphocytes in mice mimicked the increased susceptibility to high doses of *Listeria*, but these mice remained resistant to low dose infection [16]. In contrast, TNF deletion in T cells did not protect mice against LPS-induced septic shock, but reduced lethality from shock induced by SEB [16], illustrating the complexity of the role of TNF produced by T cells. Similarly, TNF produced by myeloid cells has been shown to control early stages of *Mycobacterium tuberculosis* infection with little role for T cell-derived TNF, whereas the latter was crucial for protection at later stages of infection [17].

Further underscoring the intricacies of TNF action, TNF can be expressed by T cells in a transmembrane form and produced as a soluble molecule after membrane cleavage by an ADAM family metalloprotease. Several groups utilized mice that express only transmembrane TNF, and found roles for both forms of TNF but under alternate scenarios [18–20]. Mice with only membrane TNF showed increased sensitivity to high doses of *Listeria* similar

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to TNF-/- mice and T cell TNF-conditional knockout mice, indicating that soluble TNF made by T cells was critical. However, transmembrane TNF expressed on memory T cells was sufficient for control of a secondary *Listeria* infection. Similar to the latter observation, TNF was found indispensable for control of *Francisella tularensis* live vaccine strain (LVS) that is mediated by memory CD8 T cells, and the transmembrane form was shown to be critical for this activity [21].

It is well established that TNF is pathogenic in many scenarios given the results from inhibiting TNF in patients with RA, Crohn's disease, psoriatic arthritis, and ankylosing spondylitis. However, TNF may be protective in other inflammatory diseases typified by reports of exacerbated symptoms in MS patients. How much T cell-derived TNF, and the soluble or membrane version, contributes to autoimmunity, or protection against autoimmunity, is not clear. In the case of the protective effect of TNF in neuroinflammation, the transmembrane form acting through TNFR2 has been suggested to be most important as shown by studies of mice capable of making transmembrane TNF but not soluble TNF, that were protected from developing MS-like disease in the murine model of EAE [18]. In other cases, variable effects of T cell-derived membrane vs. soluble TNF have been seen in GVHD models. Mice receiving T cells expressing only membrane TNF exhibited less severe GVHD compared to those receiving T cells that could produce soluble TNF. In contrast, the graft-versus-tumor activity of the T cells remained intact when only membrane TNF could be produced [22], again illustrating different roles for the two versions. In type I diabetes, T cells have been suggested to have a prominent role in pathogenesis, and elimination of CD8 T cells fully protects mice from the experimental disease. Indirect data has suggested that membrane TNF interacting with TNFR2 is required for islet destruction [23]. Furthermore, pathogenic Th1 clones that make soluble TNF when transferred into NOD/SCID recipients can drive autoimmune attack in the pancreas [24]. T cells are also one of the most abundant cell types in the RA synovium, comprising 30–50% of synovial tissue cells [25], but there is little understanding about how T cell derived TNF contributes to disease. Initial studies with an overexpression system of TNF in T cells showed this was sufficient to promote arthritis, wasting syndrome, and organ necrosis [26]. Experiments using knock-in mice with a deletion of the AU-rich elements in the TNF gene 3′ UTR, that results in overproduction of TNF, showed the development of chronic inflammatory arthritis and inflammatory bowel disease. Interestingly, when crossed with RAG 1-/- mice, these animals still displayed full signs of destructive arthritis, but were protected from Crohn's-like intestinal phenotype, implying a role for TNF derived from T cells in the later but not former phenotype [27].

Another variable in terms of the activity of T cell derived TNF is that the transmembrane form can be a receptor as well as a ligand for TNFR1/2, and similar to other TNF family proteins membrane TNF can "reverse" signal into the cell that bears this molecule [28]. It is not clear what is the physiological role of any signal generated by cross-linking membrane TNF, and reported activities from in vitro studies describe both anti-inflammatory and pro-inflammatory effects in T cells depending on the subset (CD4 or CD8) and depending on the aspect of response analyzed [29,30]. Anti-inflammatory effects of reverse signaling through membrane TNF have been discussed as contributing to the therapeutic effects of the approved anti-TNF biologics, such as infliximab, in particular the idea that this can induce apoptosis in cells such as monocytes/macrophages [31]. Although antibodies cross-linking membrane TNF have been reported to also promote apoptosis in T cell lines or upregulation of suppressive molecules like IL-10 [32], there is a paucity of information in this regard in primary T cells and from in vivo studies.

In summary, although we have not reviewed all of the literature that relates to T cell production of TNF, it is clear that both the membrane bound form and the soluble form can be active, but when and where they are active, in driving or protecting against autoimmune disease, or protecting against pathogens, is highly variable and not necessarily predictable.

3. Activity of TNF on naïve and effector T cell proliferation and survival

As opposed to the role of TNF made by T cells, a substantial amount of data has now shown that T cells can be the recipients of TNF activity. During the early phase of T cell receptor (TCR) triggering, TNF has been shown to be capable of enhancing TCR-dependent activation of both CD4 and CD8 T cells, including augmenting levels of IL-2R, enhancing T cell proliferation, and increasing cytokine production [7,10]. This is potentially by increasing the activation of NF-κB signaling pathways that can impact all of these functional responses [33]. As such TNF can act as a co-stimulatory molecule for T cells given that antigen recognition only weakly activates NF-κB. Importantly, it is TNF signaling through the TNFR2, and not TNFR1, that has been shown to be directly co-stimulatory to TCR-mediated T cell activation [34–37]. TNFR2-deficient T cells are less responsiveness when activated via cross-linking the TCR, and interestingly, provision of CD28 signals did not restore the response of these cells, thus suggesting a non-redundant role for TNF in promoting early T cell activity [38]. Importantly, TNFR2 signals were found to lower the threshold level of TCR signaling required for T cell activation, again consistent with the idea of supplementing NF-κB activity, that can be limiting when the TCR is engaged in isolation [38]. In line with this, a TNFR2-deficiency in mice resulted in both decreased proliferation and cytokine production in CD4 and CD8 T cells [38,39]. Similarly, TNF/TNFR2 signals in T cells were found to be critically required for effective priming, proliferation and recruitment of CD8 T cells to the sites of tumor growth in vivo, although were not required for the stronger CD8 T cell response to LCMV [40]. These are the types of observations that have also been reported for many other co-stimulatory molecules in varying scenarios, including CD28, and TNFR family members such as OX40 and CD27, where the level of antigenic stimulation and the presence of other inflammatory molecules can modulate how critical certain molecules are to the T cell response. However, it again supports the notion that TNF can be viewed as a bona fide co-stimulatory ligand for T cells. In addition, as reported with other co-stimulatory molecules [1], TNFR2 signaling in CD4 effector T cells was recently shown to increase resistance to regulatory T cell (Treg)-mediated suppression [41], and signaling into responding naïve CD4 T cells antagonized the action of TGF-β in promoting the differentiation of $Foxp3^+$ Treg cells [42]. This furthers the conclusion that TNF can play a strong role in promoting the effector arm of the T cell response.

In addition to TNF's action in driving T cell proliferation, it can also contribute to sustaining T cell survival during initiation of immune responses. Some studies have revealed TNF signaling through TNFR2 can increase expression of anti-apoptotic molecules such as Bcl-2, Bcl-xL and survivin during the early phase of T cell activation [39,43]. These observations were strengthened by finding that TNFR2-deficient mice can possess significantly reduced pools of memory T cells that is likely a consequence of poor effector T cell accumulation in the primary response [39]. Additionally, it was reported that the clinically approved TNF neutralizing antibodies led to T cell apoptosis, when CD4 T cells from the lamina propria and membrane TNF^+ intestinal macrophages from IBD patients were cultured together [44], likely indica-

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