Tumor Necrosis Factor and Its Blockade in Granulomatous Infections: Differential Modes of Action of Infliximab and Etanercept?

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Tumor necrosis factor (TNF) is a critical component of both the antibacterially protective and the inflammatory responses against infections, particularly infections with intracellularly viable microorganisms. It is, therefore, not surprising that some treatment regimens that target TNF function have resulted in an increase in complications associated with infections due to such pathogens as Mycobacterium tuberculosis, Listeria monocytogenes, and Histoplasma capsulatum; organized granuloma formation is required to keep such infections under control. However, treatment with anti-TNF monoclonal antibodies (i.e., infliximab) has been associated with a higher incidence of granulomatous infections than has treatment with a TNF receptor (TNFR) p75 immunoglobulin G–fusion construct (i.e., etanercept). Three hypotheses concerning the mode of action of these 2 agents that might explain this difference are discussed here: differential induction of apoptosis or lysis in membrane TNF–expressing macrophages and T cells, differential inhibition of signaling via TNFRp55 and TNFRp75, and different net neutralizing capacities resulting from different pharmacologic properties.

There can be no doubt that the treatment of such diseases as rheumatoid arthritis and spondyloarthropathies has been revolutionized by the introduction of TNF-targeted biological agents, including infliximab and etanercept [1–3]. However, increasing numbers of reports of infection-associated complications during TNF blockade have also highlighted the fact that TNF is a critical component in containing infections with intracellular microorganisms and that an increased rate of sometimes life-threatening complications may be the price paid for superior therapeutic efficacy [4].

TNF AND TNF-TARGETED AGENTS

TNF is a multipotent cytokine that occurs in monomeric and trimeric soluble and transmembrane forms [5]. The soluble form binds to both TNF receptors (TNFRs), p55 and p75, whereas the transmembrane form predominantly signals via TNFRp75 [6]. Infliximab is a chimeric anti-TNF monoclonal antibody, with murine variable regions and human IgG1 constant regions, that binds both monomeric and trimeric soluble TNF and transmembrane TNF [7]. Etanercept is a dimeric fusion protein consisting of the extracellular portion of TNFRp75 linked to the Fc domains of human IgG1. Etanercept binds only trimeric TNF and interacts with transmembrane TNF with reduced avidity, compared with that of infliximab [7]. Infliximab is administered at 14-day intervals by intravenous infusion, whereas etanercept requires delivery twice weekly by self-administered subcutaneous injection.

Infliximab is highly effective for the treatment of rheumatoid arthritis and steroid-refractory Crohn disease and induces long-term remissions [8, 9]; activity of infliximab against sarcoidosis and Wegener granulomatosis has also been reported [10, 11]. Etanercept is equally effective for the treatment of rheumatoid arthritis but is ineffective for the treatment of Crohn disease and, at least in the currently used dosage regimen, for the treatment of sarcoidosis [8, 12–14]. One might, therefore, speculate that, although the 2 drugs...
share a common therapeutic target, they might also differ in some aspects of their mode of action.

**ROLE OF TNF IN GRANULOMATOUS INFECTIONS**

Studies of mouse model systems have been instrumental in defining multiple checkpoints at which TNF is critical for successful containment or elimination of intracellular pathogens. Three important facets of TNF activity stand out (figure 1).

**Involvement of TNF in the recruitment of inflammatory cells.** TNF stimulates the production of such chemokines as CCL-2, -3, -4, -5, and -8 in macrophages and in T cells and induces the expression of vascular adhesion molecules (e.g., CD54), thus promoting a focused accumulation of immune cells at the site of infection [15, 16]. In TNF-deficient mice infected with *Mycobacterium tuberculosis*, granuloma formation is delayed and poorly organized, which leads to inefficient containment of infectious foci [15]. Similarly, neutralization of TNF activity after the establishment of granulomas results in their structural disintegration [17].

**Major macrophage-activating activity of TNF.** TNF increases the phagocytic capacity of macrophages and enhances the killing of intracellularly viable bacteria via the generation of reactive nitrogen and oxygen intermediates, effectively synergizing, in this respect, with IFN-γ [18]. Neutralization of TNF activity leads to resumption of mycobacterial growth within granulomas during chronic latent infection [19]. Mice deficient in TNF or TNFRp55 have dramatically increased microbial loads and die prematurely of infections with *Listeria monocytogenes*, *M. tuberculosis*, or *Histoplasma capsulatum* [15, 20–22].

**Regulation and limitation of inflammatory responses by TNF.** TNF can induce apoptosis in TNFRp55-bearing cells, thereby eliminating excessive cellular responses [19]. On the other hand, TNF can also act as a survival factor and may be involved in the maintenance of macrophage viability at the site of infection. In TNFRp55-knockout mice infected with *Mycobacterium avium*, the granuloma structure cannot be maintained, and the dysregulated, hyperinflammatory response causes the premature death of infected mice [23].

**INTRACELLULAR INFECTIONS ASSOCIATED WITH THE CLINICAL USE OF TNF-TARGETING AGENTS**

Given the critical role that TNF plays in stimulating the granulomatous inflammation necessary for the containment of intracellular infections, it is not surprising that the clinical use of TNF inhibitors is associated with an increased risk of developing infectious complications. Although it is sometimes difficult to directly compare the reported rates of infectious complications associated with the various treatment regimens, the emerging consensus is that antibody-mediated neutralization by infliximab is associated with an ~5–10-fold increased risk of reactivation of tuberculosis, whereas soluble receptor-mediated modulation of TNF function by etanercept is associated with no or only a slightly increased risk of reactivation of tuberculosis, compared with the background incidence [4, 12, 24–28]. In addition, such complications as histoplasmosis or listeriosis rarely occur among patients treated with etanercept but have been the subject of recent case reports of patients treated with infliximab [29, 30]. Given that infliximab is of great value for the treatment of Crohn disease and sarcoidosis, whereas the benefit of etanercept is limited for the treatment of these granulomatous conditions, it might be argued that...
infliximab somehow directly interferes with granuloma integrity but that etanercept predominantly neutralizes excessive inflammatory responses.

THREE HYPOTHESES REGARDING THE DIFFERENTIAL MODES OF ACTION OF INFLIXIMAB AND ETANERCEPT

**Differential induction of target cell death.** Once monoclonal antibodies, such as infliximab, have bound to membrane-anchored TNF on monocytes or lymphocytes, they can activate complement or cause antibody-dependent cellular cytotoxicity via their Fc tails [31]. In this scenario, lysed granuloma macrophages would spill *M. tuberculosis* organisms into the bloodstream, which would readily explain the relatively high occurrence of disseminated tuberculosis in infliximab-treated patients. However, no studies have directly demonstrated lysis of macrophages by infliximab in vivo.

Infliximab has been shown to cause apoptosis in monocytes from patients with Crohn disease through a caspase 3–dependent pathway, and ex vivo studies of patients treated with infliximab have demonstrated a significant increase in apoptotic CD3+ lymphocytes in the lamina propria of colonic biopsy specimens [32, 33]. It is possible that elimination of effector T cells represents the true mode of action for anti-TNF antibodies, which would also explain why anti-TNF antibodies provide superior efficacy in the treatment of Crohn disease, compared with etanercept. If T cells with specific reactivity against mycobacterial antigens express transmembrane TNF, and if anti-TNF antibodies cause the elimination of these memory cells by inducing apoptosis, reactivation tuberculosis in infliximab-treated patients would be readily explained by the loss of specifically reactive, IFN-γ–producing and macrophage-activating T cells. However, this hypothesis has not been formally demonstrated. Etanercept has not been reported to have apoptosis-inducing activity.

**Differential inhibition of TNF signaling.** On the basis of the differential binding avidities of infliximab and etanercept for soluble versus transmembrane TNF, it can be predicted that infliximab inhibits both TNFRp55– and TNFRp75-mediated events, whereas etanercept leaves TNFRp75-mediated signaling at least partially intact [7, 34]. In support of this hypothesis, TNF-deficient mice that were made transgenic for only the transmembrane form of TNF retained substantial resistance against mycobacterial challenge infections, which would argue that transmembrane TNF signaling via TNFRp75 may provide sufficient residual protective immunity under certain experimental conditions [35]. Moreover, studies of murine models of autoimmune diseases have convincingly shown that TNFRp55 signaling is associated primarily with tissue-damaging events, whereas TNFRp75 signaling supports immunoregulatory functions [36].

**Differential net blockade of TNF bioactivity.** Because of its high association rate and very low dissociation rate, infliximab binds to TNF quickly and irreversibly. In contrast, etanercept has both fast on and fast off binding kinetics and sheds ~50% of soluble TNF and 90% of transmembrane TNF only 10 min after binding [7]. Infliximab, therefore, completely neutralizes TNF bioactivity, whereas freely diffusing etanercept might be considered to redistribute bioavailable TNF from sites of production to sites of lower concentration. If one assumes that chronic inflammatory diseases differ in terms of their absolute requirement for TNF bioactivity for disease symptoms to develop, this hypothesis might explain why infliximab is therapeutically superior to etanercept for the treatment of such conditions as Crohn disease and sarcoidosis but is equally effective for the treatment of rheumatoid arthritis and spondyloarthropathies.

This hypothesis would imply that, in the context of granulomatous infections, infliximab disrupts granuloma integrity, because complete neutralization of TNF will result in a complete lack of the inflammatory cell recruitment necessary to offset cellular turnover. Etanercept, on the other hand, could be envisioned to partially preserve granuloma integrity, because it would allow the shedding of bioactive TNF (removed from sites of production, such as the rheumatoid joint) in other tissues.

There are no data on the actual amount of TNF that causes tissue-damaging inflammation and pain in the context of rheumatoid arthritis. Likewise, there are no in vivo data on the minimum amount of TNF required to provide sufficient macrophage activation and granuloma maintenance in the context of intracellular infections. Hypothetically, 70%–90% blockade of TNF bioactivity might leave sufficient antimicrobial effector mechanisms intact to prevent reactivation of latent tuberculosis, while adequately relieving symptoms of disease in patients with rheumatoid arthritis (figure 2). In a futuristic scenario, determination of TNF levels in various tissues (e.g., by biosensors) may allow for more-precise titration of the level of bioactive TNF in different tissues (e.g., by a chip-controlled minipump administering TNF-targeted agents). As a consequence, excessive inflammatory responses (e.g., in the arthritic joint) may be inhibited, while baseline capacities to combat infections (e.g., in the lung) are preserved.

**SUMMARY**

TNF is a critical regulatory cytokine that drives both protective and inflammatory responses to *M. tuberculosis* infection. Therefore, interfering with TNF activity is a double-edged sword: although low tissue levels of bioactive TNF are beneficial in patients with such conditions as rheumatoid arthritis, they may not be sufficient to maintain the continuous supply of effector cells into infectious foci, thus compromising cell-mediated immunity to tuberculosis. The differential safety and efficacy profiles of infliximab and etanercept have taught researchers that
Figure 2. “TNF-o-meter” illustrating a hypothetical correlation of disease activity for rheumatoid arthritis or tuberculosis with the absolute level of bioactive TNF. High amounts of TNF (central gray-shaded column) cause symptoms in rheumatic joints (100% disease [left scale]) but provide containment of mycobacterial infection within granulomas (0% likelihood of reactivation [right scale]). The use of TNF-targeted agents may reduce TNF bioactivity to a level that is sufficient to sustain sufficient protective antimycobacterial immunity (green/yellow area [right scale]) yet is adequate for pain relief (green/yellow area [left scale]). The therapeutic window (overlapping green and yellow shades on the left and right scales) is defined by the differential absolute tissue levels of TNF necessary for disease activity in patients with rheumatoid arthritis, compared with microbial containment in patients with tuberculosis.

the future development of TNF-targeted drugs will need to focus on different aspects of TNF biology in different diseases. To fine-tune the mode of action of biological agents during anti-inflammatory therapy, threshold levels for TNF bioactivity in situ, differential receptor-binding kinetics, and possible induction of target cell death will have to be investigated in more detail.

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References


