Why Does Tumor Necrosis Factor Targeted Therapy Reactivate Tuberculosis?

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ABSTRACT. Treatment of chronic inflammatory conditions, such as rheumatoid arthritis and Crohn's disease, with tumor necrosis factor (TNF) targeted biological therapies is associated with an increased risk of infectious complications, especially tuberculosis (TB). Clinical studies have revealed that monoclonal anti-TNF antibodies (e.g., infliximab) more frequently reactivate TB than a TNF receptor p75 immunoglobulin fusion construct (etanercept). Experimental studies in mice have shown TNF to be an essential component of protective granuloma formation. Based on these studies and the known pharmacological properties of the 2 prototype TNF targeted biologic agents, this review discusses 3 hypotheses that might explain the unpredicted differential risk of infectious complications: differential induction of target cell death, differential TNF receptor signaling, and differential net inhibition of TNF bioavailability. (J Rheumatol 2005;32 Suppl 74:35-39)

Key Indexing Terms:
TUMOR NECROSIS FACTOR
APOPTOSIS
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CROHN'S DISEASE

INTRODUCTION
A number of recent reports have drawn attention to infectious complications associated with the use of tumor necrosis factor (TNF) targeted biological therapies1,2. In particular, increased reports of reactivation tuberculosis (TB) are of concern3,4. Although it is almost impossible to directly compare complication rates in the different clinical trials, there is substantial evidence that treatment with anti-TNF antibodies (e.g., infliximab) bears an increased risk of 5 to 10-fold of reactivating TB, compared to other treatments in the respective study populations5,6. In contrast, treatment with a TNF receptor p75 immunoglobulin fusion construct (etanercept) does not appear to lead to a higher incidence of TB7. Since both reagent classes share the same therapeutic target (neutralization of TNF activity), it is intriguing to speculate that they must differ in some other mode of action that might explain this differential frequency of infectious complications.

TNF AND TNF TARGETED BIOLOGICS
TNF is a multipotent cytokine that occurs in a soluble and transmembrane form. The soluble form binds to both TNF receptors (p55 and p75), whereas the transmembrane form predominantly signals via TNFRp758. Infliximab is a chimeric anti-TNF monoclonal antibody with murine variable regions and human IgG1 constant regions that binds soluble TNF and transmembrane TNF9. Infliximab needs to be administered intravenously every 2 weeks. Diseases for which infliximab is effective include Crohn's (CD), psoriasis, sarcoidosis, rheumatoid arthritis (RA), and the spondyloarthropathies (SpA). Etanercept is a dimeric fusion protein consisting of the extracellular portion of the human p75 TNF receptor linked to the Fc domains of human IgG1 that antagonizes soluble TNF and transmembrane TNF9. Infliximab needs to be administered intravenously every 2 weeks. Diseases for which infliximab is effective include Crohn's (CD), psoriasis, sarcoidosis, rheumatoid arthritis (RA), and the spondyloarthropathies (SpA). Etanercept is a dimeric fusion protein consisting of the extracellular portion of the human p75 TNF receptor linked to the Fc domains of human IgG1 that antagonizes soluble TNF and transmembrane TNF9. Infliximab needs to be administered intravenously every 2 weeks. Diseases for which infliximab is effective include Crohn's (CD), psoriasis, sarcoidosis, rheumatoid arthritis (RA), and the spondyloarthropathies (SpA). Etanercept is administered subcutaneously twice weekly. It is therapeutically effective in RA and SpA, but not in CD or sarcoidosis.

COMMON MODE OF ACTION: NEUTRALIZING TNF ACTION
Granuloma formation is the hallmark of mycobacterial infections and represents an effort by the host to contain persisting viable microorganisms (Figure 1)10. Following ingestion of Mycobacterium tuberculosis in alveolar macrophages, TNF, among other proinflammatory cytokines, triggers a steady influx of granulocytes and monocytes into the alveolar and interstitial spaces11. TNF is necessary to fully mature dendritic cells for antigen-presentation in the local lymph node and thus primes and amplifies the specific T cell response12. Recruitment of monocytes and circulating antigen-specific T cells into the site of infection is made possible by TNF action on the vascular endothelium (e.g., induction of adhesion molecules such as ICAM-1) and the capacity of TNF to...
establish chemokine gradients [e.g., for chemokine CC motif ligand 2 (CCL2), CCL3, CCL5]13,14. Effector T cells then produce both TNF and interferon-γ (IFN-γ) within the lesion to drive the macrophage enzymatic machinery that elaborates reactive oxygen and nitrogen intermediates with potent antimicrobial activity15,16. Under the influence of TNF and T cell derived IFN-γ, mononuclear cells accumulate to form a highly structured granuloma. Here, TNF and IFN-γ activate mycobactericidal pathways within macrophages and regulate excessive inflammation by inducing apoptosis of T cells. C. Macrophage and T cell derived TNF is necessary to continuously orchestrate the recruitment of mononuclear cells into granulomas in order to maintain effective containment of mycobacterial foci of infection.

Figure 1. TNF acts at multiple steps in protective granuloma formation in response to M. tuberculosis infection. A. TNF derived from infected alveolar macrophages initially induces recruitment of a mixed cellular alveolar and interstitial infiltrate. B. Under the influence of TNF and T cell derived IFN-γ, mononuclear cells accumulate to form a highly structured granuloma. Here, TNF and IFN-γ activate mycobactericidal pathways within macrophages and regulate excessive inflammation by inducing apoptosis of T cells. C. Macrophage and T cell derived TNF is necessary to continuously orchestrate the recruitment of mononuclear cells into granulomas in order to maintain effective containment of mycobacterial foci of infection.

Net neutralization. Infliximab binds TNF in a fast and irreversible fashion in vitro. In contrast, etanercept has both high on- and high off-binding kinetics, shedding about 50% of soluble TNF and 90% of transmembrane TNF only 10 minutes after binding9. Once bound to infliximab, TNF is therefore completely neutralized, whereas TNF bound to etanercept may be shed in a bioavailable form at sites of infected animals21,25,26. Moreover, neutralization of TNF during chronic latent tuberculosis is followed by resumption of bacterial replication within granulomatous lesions27-29.

Neutralization and binding studies with exogenously added TNF in vitro have demonstrated that both infliximab and etanercept efficiently block TNF bioactivity9,30. In terms of infectious complications, one would therefore predict that all TNF targeted biologicals should adversely affect the risk of infection, particularly with intracellularly viable microorganisms that require granuloma formation for their containment.

DIFFERENTIAL MODE OF ACTION: 3 HYPOTHESES

Why then would some TNF-neutralizing agents be more fraught with infectious complications than others? Three hypotheses may be considered that involve differential modes of action of the 2 classes of TNF targeted biologics, monoclonal antibodies and soluble TNF receptor constructs (Figure 2).

TNF signaling. Infliximab binds both soluble and transmembrane TNF, whereas etanercept has a 4-fold lower binding affinity for transmembrane TNF9. This implies that infliximab inhibits both TNFRp55 and TNFRp75 mediated events, whereas etanercept may leave TNFRp75 mediated signaling at least partially intact. Studies in murine models of inflammatory and autoimmune disorders have highlighted that TNFRp55 signaling is predominantly associated with the proinflammatory tissue-damaging sequelae of TNF action, whereas TNFRp75 signaling mostly supports immunoregulatory functions and may, indeed, suppress inflammatory events31.

In the mouse model of L. monocytogenes infection, the death domain of the TNFRp55 was unequivocally shown to be the essential signal-transducing component for initiating bactericidal pathways32,33, whereas mice lacking the TNFRp75 behaved identically to wild-type mice34. Evidence is only now accumulating in infection models with M. tuberculosis that mice with only the transmembrane form of TNF are still relatively resistant against mycobacterial challenge infections35. This would argue that transmembrane signaling via TNFRp75, possibly preserved during the use of etanercept, may provide sufficient residual protective immunity against TB to prevent reactivation, at least under certain experimental conditions.

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Infliximab has been shown to cause apoptosis in monocytes and lamina propria T cells from patients with CD30,37. It is possible that elimination of effector T cells represents an additional mode of action for anti-TNF antibodies, explaining why they provide superior efficacy in CD over 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 TB in infliximab treated patients might tentatively be the consequence of the loss of specifically reactive, IFN-γ-producing and macrophage-activating T cells. This hypothesis, however, has not been formally confirmed. Indeed, analyses from tissue biopsies following anti-TNF treatment in humans and mice are conflicting: apoptosis was found to be increased in M. tuberculosis infected mice27, but was apparently reduced in granulomas following infliximab treatment in patients3.

The relative importance of apoptosis in the defense against M. tuberculosis infection is controversial. In vitro studies demonstrating that highly virulent mycobacterial strains can evade apoptosis in alveolar macrophages have argued that this may represent an effective evasion mechanism on the part of the mycobacterium38,39. In addition, some studies show decreased viability of mycobacteria in apoptotic cells40,41, particularly if induction of apoptosis is accompanied by the presence of granulysin or other mycobactericidal peptides17,42, but others have failed to confirm this finding43. Indeed, as long as mycobacteria are not effectively killed by the apoptotic machinery, the uptake of apoptotic cells by bystander macrophages is a process that causes little inflammation and may be thought of as a Trojan horse mode of “silent entry.” In corroboration of the latter view, there is a lot of macrophage apoptosis even in TNFRp55-deficient mice without any beneficial effect on mycobacterial killing26.

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There are no data on the actual amount of TNF that causes tissue-damaging inflammation and pain in the context of RA. Similarly, there are no in vivo data on the minimal amount of TNF required in the context of intracellular infections to provide sufficient macrophage activation and granuloma maintenance. Hypothetically, 70-90% blockade of TNF bioactivity might leave sufficient antimicrobial effector mechanisms intact to prevent reactivation of latent TB, while still adequately relieving disease symptoms in RA.

Target cell death. Monoclonal antibodies such as infliximab — after binding to membrane-anchored TNF on monocytes or lymphocytes — are known to activate complement or cause antibody-dependent cellular cytotoxicity via their Fc tails36. In the context of TB, lysed granuloma macrophages would release mycobacteria into the bloodstream, providing a possible explanation for the relatively high occurrence of disseminated TB in infliximab-treated patients. However, no studies have directly demonstrated target cell lysis by infliximab.

Figure 2. Differential modes of action of infliximab and etanercept. A. Soluble TNF (small rectangles, released from cell membrane at the bottom) preferentially binds to TNF-Rp55 (dark trimeric tubes on top of cell membrane), transmembrane TNF (large rectangles) predominantly binds to TNF-Rp75 (light trimeric tubes). B. Etanercept preferentially neutralizes soluble TNF, leaving TNF-Rp75 signaling largely intact. C. Infliximab neutralizes both soluble and transmembrane TNF forms. D. Infliximab binds to transmembrane TNF and via complement or antibody-dependent cellular cytotoxicity (*) initiates lysis of target cells. Alternatively, infliximab induces reverse signaling in transmembrane TNF-bearing cells, causing apoptosis.

lower concentration. In the context of TB, this would imply that infliximab disrupts granuloma integrity, because 100% neutralization of TNF will invariably result in a severely diminished inflammatory cell recruitment, so that the high turnover of cells within the dynamic granuloma can no longer be maintained. Also, 100% blockade will substantially decrease macrophage activation, causing M. tuberculosis organisms to regrow within the granuloma. Etanercept, on the other hand, could be envisioned to partially preserve granuloma integrity because it would allow redistribution of bioactive TNF from sites of production, such as the rheumatoid joint, into other tissues where overall TNF concentrations are low. TNF bioactivity therefore may not be entirely blocked, preserving residual antimicrobial functions of the macrophage in granulomatous lesions.

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The relative importance of any apoptosis-promoting properties of anti-TNF antibodies remains to be determined in further studies. Etanercept has not been reported to have apoptosis-inducing activity.

CONCLUSION

The differential risk profile of the 2 most commonly used TNF targeted biologicals, infliximab and etanercept, have highlighted the gaps in our understanding of how they truly work in vivo. With respect to TB, animal models are available that can help identify which hypothesis concerning the mode of action is most likely to explain the differential efficacy and safety profile. It is to be hoped that from this renewed alliance of clinical and basic research efforts a new generation of TNF targeted drugs may be developed that minimize the risk of infection while preserving their antiinflammatory potency.

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REFERENCES