

# Tumour necrosis factor antagonists: structure, function, and tuberculosis risks

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Our understanding of the infection risks presented by tumour necrosis factor (TNF) antagonists has continued to evolve in the 10 years since these drugs were first introduced. Several recent studies have confirmed the increased risk of tuberculosis posed by TNF antibodies compared with soluble TNF receptor, particularly with regard to reactivation of latent infection. Structural and functional differences seem to account for this finding. This Review examines the potential relations between target specificity, stoichiometry, and binding kinetics of TNF blockers and their associated risk of infection. Clinical strategies for prevention and management of tuberculosis in patients treated with TNF blockers may be improved based on our evolving understanding of these differences.

## Introduction

This year marks the tenth anniversary of the approvals of the first tumour necrosis factor (TNF)-alpha antagonists for the treatment of chronic inflammatory conditions. Two classes of antagonists, monoclonal antibody and soluble receptor, have proven to be highly effective treatments for rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis, and juvenile rheumatoid arthritis. However, as clinical experience with these drugs has grown, differences between them, regarding efficacy in granulomatous inflammatory conditions and risk of granulomatous infections—especially tuberculosis—have become apparent. Our understanding of this subject has continued to evolve since it was reviewed in 2003.<sup>1</sup> This Review focuses on recent publications that elucidate the structure–function relation of the TNF blockers in the context of tuberculosis, and that inform tuberculosis prevention and management in this susceptible patient population.

## TNF biology

Members of the TNF and TNF receptor (TNFR) superfamily are important regulators of immune cell proliferation, survival, differentiation, and apoptosis.<sup>2–4</sup> TNF is first produced as a transmembrane protein (tmTNF), which then is cleaved by a metalloproteinase to a soluble form (sTNF).<sup>5–7</sup> Biological activity results from the association of three monomers to form trimeric TNF, which then binds to cell-surface TNFR1 or TNFR2, leading to receptor oligomerisation.<sup>8–10</sup>

TNFR1 and TNFR2 have shared and unique activities. Both receptors can signal through anti-apoptotic and proinflammatory pathways; TNFR1 can also signal through death domain caspase-dependent pathways leading to apoptosis.<sup>4</sup> Experiments with R1 and R2 knockout mice indicate that TNFR1 is necessary for defences against bacterial infection, whereas TNFR2 might have a role in downregulating TNF-driven inflammatory signals.<sup>11,12</sup> These differences might confer specific activities to the two forms of TNF, since TNFR2 is fully activated by tmTNF but not by sTNF.<sup>13</sup>

TNF has a central role in the initial host response to infection.<sup>3,14</sup> In tuberculosis, it results in macrophage

activation, cell recruitment, granuloma formation, and maintenance of granuloma integrity.<sup>15–20</sup> Mice lacking the gene for TNF or TNFR1, or treated with an anti-TNF monoclonal antibody, fail to contain the infection after challenge with *Mycobacterium tuberculosis*.<sup>16,18</sup> Other studies have implicated TNF, TNFR1, and TNFR2 as being important in murine defences against other intracellular pathogens such as *Listeria monocytogenes* and *Salmonella typhimurium*.<sup>11,21–23</sup>

Several studies have examined whether the closely related cytokine lymphotoxin (previously known as TNFβ) has a distinct role in antimycobacterial host defences. Active lymphotoxin is a heterotrimer composed of α and β subunits. Mice deficient in lymphotoxin α or β show increased bacillary burdens in lung after *M tuberculosis* challenge.<sup>24,25</sup> Mice lacking both TNF and lymphotoxin are highly susceptible to challenge with attenuated mycobacteria such as *Mycobacterium bovis* BCG.<sup>26,27</sup> However, lymphotoxin alone seems insufficient to support granuloma formation in TNF-deficient mice,<sup>18</sup> indicating distinct roles for these cytokines in antimycobacterial host defences.

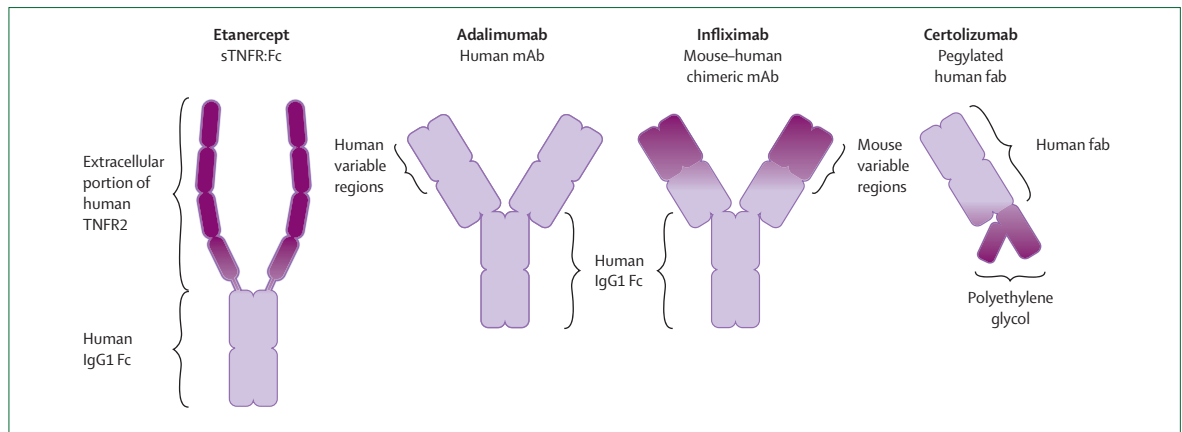
Several studies have also examined whether tmTNF is sufficient for protection against granulomatous pathogens. Transgene expression of tmTNF has been shown as sufficient to restore defences against *M bovis* BCG in mice lacking both TNF and lymphotoxin, and to partly restore defences against virulent *M tuberculosis*.<sup>20,28,29</sup> However, mice expressing only tmTNF ultimately succumb to *M tuberculosis* infection.<sup>20</sup> tmTNF has also been shown to partly protect against *L monocytogenes*.<sup>30–32</sup>

Chronic *M tuberculosis* infection after low-dose aerosol infection of mice has been studied as a model of human latent infection, since these animals have stable bacterial burdens and relatively unimpaired survival. Treatment of chronically tuberculosis-infected mice with a TNF-neutralising antibody resulted in a ten-fold increased bacillary load, compromised granuloma structure, and shortened survival.<sup>17</sup> These experiments therefore confirm a requirement for TNF for granuloma maintenance as well as formation. They also indicate distinct and shared activities of the different forms of TNF in the host response to infectious agents, thereby

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**Figure 1: Structures of the TNF antagonists**

Fab=fragment, antigen binding. mAb=monoclonal antibody. TNFR2=tumour necrosis factor receptor 2. sTNFR=soluble tumour necrosis factor receptor.

supporting the concept that selective inhibition of sTNF by therapeutic TNF blockers might carry reduced infection risk.

### TNF antagonists

There are currently three approved TNF monoclonal antibodies: infliximab, adalimumab, and certolizumab pegol (figure 1). Infliximab is a chimeric monoclonal antibody composed of a human IgG1 constant region and a murine variable region. Adalimumab is a humanised monoclonal antibody, with both human IgG1 constant and variable regions. Certolizumab pegol is a pegylated, humanised monoclonal anti-TNF Fab' (fragment, antigen binding) fragment. Infliximab and adalimumab are approved for the treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and Crohn's disease.<sup>33,34</sup> The pivotal trials of these antibodies in patients with rheumatoid arthritis showed 40–50% of patients achieved an ACR50 response (a 50% improvement in the American College of Rheumatology composite score); in patients with Crohn's disease, a similar proportion entered clinical remission.<sup>33,34</sup> Infliximab is also approved for ulcerative colitis, and could be effective in sarcoidosis (although responses do not appear as complete as in Crohn's disease).<sup>35–37</sup> Certolizumab was recently licensed for the treatment of Crohn's disease, and seems to be effective for rheumatoid arthritis.<sup>38–40</sup> Infliximab is administered by intravenous infusion, usually at 4–6-week intervals, typically producing maximum and minimum blood concentrations of more than 80 µg/mL and less than 5 µg/mL, respectively.<sup>41</sup> Adalimumab and certolizumab are administered by subcutaneous injection, usually every other week. Blood concentrations of 5–10 µg/mL have been reported for adalimumab.<sup>42</sup>

One soluble TNFR, etanercept, is currently in clinical use. It consists of two extracellular domains of the human TNFR2 fused to the Fc fragment of human IgG1. Unlike the antibodies, etanercept binds both TNF and

lymphotoxin (including lymphotoxin-α homotrimers). Etanercept is approved for the treatment of rheumatoid arthritis, psoriatic arthritis, psoriasis, juvenile rheumatoid arthritis, and ankylosing spondylitis.<sup>43</sup> ACR50 response rates of 40–50% were reported in pivotal trials in patients with rheumatoid arthritis.<sup>43</sup> Etanercept seems to be ineffective for the treatment of granulomatous inflammatory conditions such as Crohn's disease or sarcoidosis.<sup>44,45</sup> It is administered by subcutaneous injection, usually once or twice weekly, producing blood concentrations of 1–2·4 µg/mL.

### TNF antagonists and granulomatous infections

Clinical studies of the effect of TNF antagonists on granulomatous infections face several distinct challenges. The rarity of these infections in North America or Europe has prevented their analysis as an endpoint in prospective randomised controlled clinical trials. A meta-analysis of pooled randomised placebo-controlled trials and extension studies of etanercept, for example, did not detect any effect of this treatment on tuberculosis compared with expected rates in these regions.<sup>46</sup> This setback forces reliance on non-randomised study designs, such as registries and voluntary reporting systems, which are inherently less reliable. Only a fraction of events are reported to voluntary systems such as the Adverse Event Reporting System (AERS) of the US Food and Drug Administration (FDA). Reports can be incomplete, lacking relevant data such as underlying medical conditions and concurrent medications. Prescribing bias might arise because of reimbursement practices in different patient populations.

Many of the infections vary by country or region. Notifications for tuberculosis per 100 000 person-years, for example, occur at a rate of 140 in Romania, 41 in Bulgaria, and 18 in Spain, but only five in Sweden, the USA, or Canada.<sup>47,48</sup> Other infections, such as histoplasmosis and coccidioidomycosis, occur within specific regions, and do not require notification of

public-health officials.<sup>49</sup> Regional differences in prescribing practices could therefore easily be misinterpreted as differences in the biological effects of treatment.

Finally, the natural history of many of these infections is complicated by the existence of long periods of clinical latency, during which the infection is contained but not eradicated by the host response. This is particularly true for tuberculosis, which can occur immediately following *M tuberculosis* infection, or which can reactivate after remaining clinically latent for decades (figure 2). There is no gold standard test for latent tuberculosis infection, since current tests do not adequately distinguish between bona fide persistence capable of reactivation and immunological memory in instances in which the infection has been eradicated by the host response.<sup>50</sup> These tests can also be falsely negative in people with inflammatory conditions such as rheumatoid arthritis, either because of the underlying disease or its treatment.<sup>51</sup> Progression of recent tuberculosis infection cannot reliably be clinically distinguished from reactivation, particularly in immunocompromised patients. As a result, differential effects of particular therapies on different stages of *M tuberculosis* infection might easily be obscured.

These challenges notwithstanding, several studies have examined the differential effect of TNF blockers on tuberculosis, and have reached rather similar conclusions. The two largest studies were done in the USA, the region of lowest incidence. In 2004, we published a study of granulomatous infections associated with infliximab or etanercept reported to FDA AERS from January, 1998, through September, 2002.<sup>52</sup> The report was corrected shortly thereafter, to remove cases of tuberculosis in European infliximab-treated patients that had been inadvertently included.<sup>53</sup> Results were analysed on a per-treated-patient basis. The main findings were that compared with etanercept, infliximab was associated with two-fold to seven-fold greater risks of tuberculosis, coccidioidomycosis, and histoplasmosis (table 1), and shorter time to tuberculosis onset (17 vs 48 weeks).

No subsequent studies have equalled the 2004 report in terms of numbers of cases or statistical power. However, other smaller studies, with alternative data collection methods, have proven invaluable in helping to confirm that the study's findings were not solely caused by reporting biases. One report, by Bergstrom and colleagues,<sup>54</sup> examined the risk factors for coccidioidomycosis in a retrospective analysis of 985 patients with rheumatoid, juvenile, psoriatic, or reactive arthritis living in southwestern USA. The study found that the risk of coccidioidomycosis attributable to infliximab was five-fold higher than that of other antirheumatic drugs ( $p < 0.01$ ), a value very close to that indicated by FDA AERS data (6.4-fold).

In 2006, Brassard and co-workers<sup>55</sup> reported a nested case-control study that examined tuberculosis risk associated with treatment of rheumatoid arthritis by use

of a US-based pharmaceutical claims database that included 112 300 patients with rheumatoid arthritis. The highest risk of tuberculosis was attributable to corticosteroids (adjusted rate ratio 1.7, 95% CI 1.3–2.2), followed by infliximab (1.6, 1.0–2.6), and then by etanercept (1.2, 0.9–1.8; table 2). The time to tuberculosis onset distributions of infliximab and etanercept were strikingly similar to those reported to the FDA AERS (table 3). However, the rate at which tuberculosis occurred in the entire rheumatoid arthritis cohort was more than five times greater than expected, possibly because of misclassification of latent tuberculosis infection as active disease.<sup>68,69</sup> The resulting dilution of true tuberculosis cases might have reduced the apparent risks of immunosuppressive therapies in this study.

Two European studies are of interest regarding experience with adalimumab. A case-control study presented in 2006 used the French RATIO (Recherche

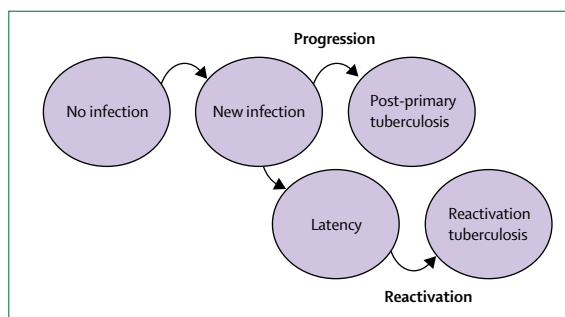


Figure 2: Progression of *M tuberculosis* infection

	Infliximab	Etanercept	I:E	p value*
Aspergillosis	8.63 (17)	6.19 (7)	1.39:1	0.243
Candidiasis	10.15 (20)	5.31 (6)	1.91:1	0.061
Bartonellosis	0.51 (1)	0 (0)	n/a	0.563
Coccidioidomycosis	5.58 (11)	0.88 (1)	6.34:1	0.013
Cryptococcosis	5.08 (10)	7.08 (8)	0.72:1	0.179
Histoplasmosis	18.78 (37)	2.65 (3)	7.09:1	<0.0001
Legionellosis	0.51 (1)	0 (0)	n/a	0.563
Leprosy	0.51 (1)	0 (0)	n/a	0.563
Listeriosis	8.63 (17)	0.88 (1)	9.81:1	0.0006
Non-tuberculosis mycobacterioses	11.17 (22)	6.19 (7)	1.80:1	0.066
Nocardiosis	3.55 (7)	0.88 (1)	4.03:1	0.090
Pneumocystosis	0.51 (1)	0 (0)	n/a	0.563
Salmonellosis	0 (0)	1.77 (2)	n/a	0.031
Toxoplasmosis	2.03 (4)	0 (0)	n/a	0.101
Tuberculosis	53.81 (106)	28.32 (32)	1.90:1	<0.0001

n/a=not applicable. Data are case rate per 100 000 treated patients (number of cases). Case rates per 100 000 treated patients were calculated on the basis of 197 000 patients treated with infliximab and 113 000 treated with etanercept, as reported by the manufacturer. I:E indicates crude case rate ratio (infliximab to etanercept). \*Significance was determined by Poisson analysis. Adapted from reference 53. Copyright 2004 by University of Chicago Press.

Table 1: Granulomatous infections in US patients treated with infliximab or etanercept

	Case rate per 1000 patient-years (number of cases)			I:A:E ratio
	Infliximab	Adalimumab	Etanercept	
USA (FDA AERS, 1998–02) <sup>53</sup>	0.5* (106)	..	0.3* (32)	1.9:n/a:1
USA (Pharmetrics, 1998–03) <sup>55</sup>	3.1 (19)	..	2.3 (32)	1.3:n/a:1
USA (NDB, 1998–99) <sup>57</sup>	0.7 (4)	..	0 (0)	n/a
UK (BSRBR, 2001–05) <sup>58</sup>	1.5 (7)	0.9 (1)	0.5 (2)	3.0:1.8:1
Sweden (ARTIS, 1999–04) <sup>59</sup>	1.5 (9)	..	0.8 (4)	1.8:n/a:1
Germany (RABBIT, 2001–03) <sup>60</sup>	3.1 (1)	..	0 (0)	n/a
Spain (BIOBADASER, 2000–01) <sup>61,62</sup>	15.0 (17)	0 (0)	0 (0)	n/a
Spain (BIOBADASER, 2002–04) <sup>63</sup>	3.9 (2)	0 (0)	0 (0)	n/a
Portugal (1999–05) <sup>64</sup>	17.5* (8)	23.0* (4)	3.0* (1)	5.8:7.7:1
France (RATIO, 2004–06) <sup>65,66†</sup>	.. (18)	.. (17)	.. (2)	5.9:14.6:1

Patients treated with more than one TNF blocker were excluded. BIOBADASER data are separated into two entries as after 2002, policies were put into place for tuberculin skin testing and isoniazid prophylaxis. ..=not reported. ARTIS=Anti-Rheumatic Treatment in Sweden. BIOBADASER=Spanish Society of Rheumatology Database on Biologic Products. BSRBR=British Society for Rheumatology Biologics Register. FDA AERS=US Food and Drug Administration Adverse Event Reporting System. I:A:E=infliximab to adalimumab to etanercept ratio. n/a=not applicable. NDB=National Databank for Rheumatic Diseases. RABBIT=Rheumatoid Arthritis–Observation for Biologic Therapy. RATIO=Recherche sur Anti-TNF et Infections Opportunistes. \*Cases per 1000 treated patients. †The study authors calculated the relative risks but did not publish the case rate. Reproduced with permission from reference 56. Copyright 2008 UpToDate Inc. For more information visit <http://www.upToDate.com>.

**Table 2: Summary of studies examining tuberculosis risk in patients with rheumatoid arthritis treated with infliximab or etanercept<sup>65</sup>**

	Infliximab	Etanercept	I:E
Wallis et al (AERS) <sup>52,53</sup>	17 weeks (248)	48 weeks (39)	1:2.8
Wolfe et al (NDB) <sup>57</sup>	21 weeks (4)	..	n/a
Keane et al (AERS) <sup>57</sup>	12 weeks (70)	..	n/a
Askling et al (ARTIS) <sup>59</sup>	19 weeks (11)	32 weeks (6)	1:1.7
Brassard et al (PharMetrics) <sup>55</sup>	17 weeks (19)	79 weeks (32)	1:4.6
Total (weighted mean)	16.2 weeks	59.6 weeks	1:3.7

Data are median time to onset (number of cases). Patients treated for any indication are included. AERS=Adverse Event Reporting System (US Food and Drug Administration). ARTIS=Anti-Rheumatic Treatment in Sweden. I:E=infliximab to etanercept ratio. NDB=National Databank for Rheumatic Diseases. RATIO=Recherche sur Anti-TNF et Infections Opportunistes.

**Table 3: Time to onset of tuberculosis after initiation of TNF antagonist therapy**

sur Anti-TNF et Infections Opportunistes) registry to examine infectious complications of TNF blockade.<sup>65,66</sup> This study identified 37 tuberculosis cases during 2.5 years: 18 associated with infliximab, 17 with adalimumab, and two with etanercept. Relative to etanercept, the odds ratios of tuberculosis risk for adalimumab and infliximab were 14.6 (95% CI 1.7–129.0) and 5.9 (0.7–47.0), respectively. Although the 95% CIs in this analysis are wide, the results indicate that the risk of tuberculosis attributable to adalimumab is substantially greater than that for etanercept, and similar to the risk attributable to infliximab.

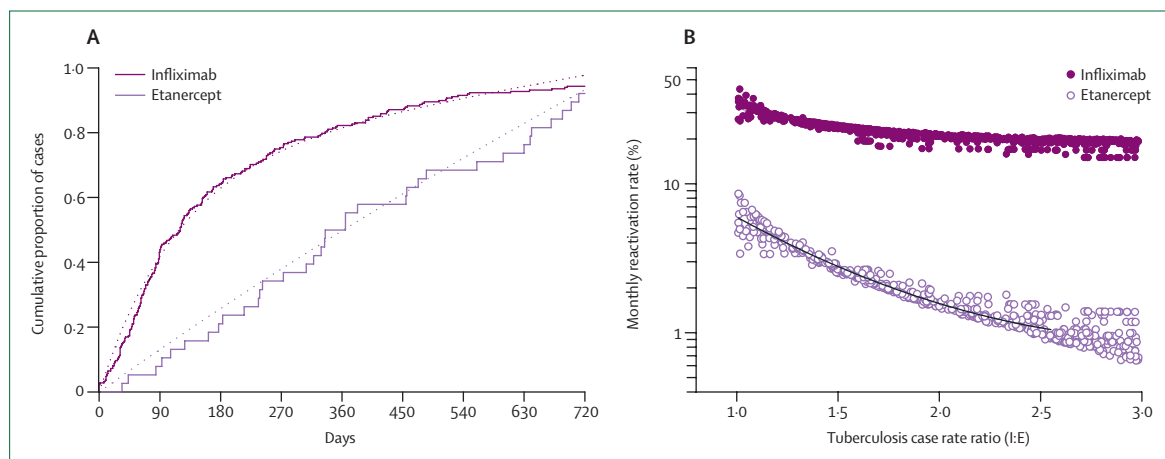
A study reviewed the Portuguese experience with TNF blockers in 960 patients treated between 1999 and 2005.<sup>64</sup> It identified 13 tuberculosis cases: eight in 456 patients treated with infliximab (18 cases per 1000 patients), four

in 171 treated with adalimumab (23 cases per 1000 patients), and one in 333 treated with etanercept (three cases per 1000 patients). Poisson analysis of these data indicates a probability of only 0.013 of the etanercept results arising from a population of the two antibodies combined. The unadjusted risk of tuberculosis associated with TNF antibodies was 6.4-fold greater than with etanercept.

These data, and that of other small studies,<sup>57–59,61–63,67</sup> are summarised in table 2 and table 3. The trends across these studies indicate reduced risk and delayed onset for etanercept-associated tuberculosis relative to TNF monoclonal antibodies. These twin observations provide a potentially very powerful insight into the cause of tuberculosis during TNF blockade, since the clustering of excess cases shortly after starting anti-TNF treatment is consistent with reactivation. By contrast, tuberculosis cases caused by progression of new infection would be anticipated to occur at random throughout treatment.

I recently examined the importance of these two observations by hidden Markov modelling.<sup>70</sup> Markov models describe transitions among clinical states, such as the occurrence of tuberculosis (figure 2). They can also contain hidden states that cannot be observed directly, but that are instead revealed by analysis of observable states. The goal of the modelling exercise was to identify rates of new infection, latent infection, progression, and reactivation that best reproduced reported time to onset distributions and case rates. The model was solved using iterative methods. The analysis took advantage of the reduced variability across studies of time to tuberculosis onset, to which was added 600 random simulations of tuberculosis case rates that reflected the full range of reported values. The results of a typical simulation are illustrated in figure 3. The close approximation of the observed and modelled time to onset distributions (solid and dotted lines, respectively) indicate a good fit of this particular model; its case rates, which are not shown, were within 2% of target values.

Two key findings emerged from this analysis. First, modelling showed the rate of reactivation of latent tuberculosis infection by infliximab to be more than 20% per month, 12.1-fold greater than that of etanercept ( $p < 0.001$ ). The difference in reactivation rates between the two drugs was largely unaffected by their respective tuberculosis case rates (figure 3). The high monthly rate of reactivation of infliximab accounted for the excess tuberculosis cases that occur shortly after treatment is started, even though the number of patients with latent infection capable of reactivation was low (150 cases per 100 000 population). Over 2 years, the cumulative number of cases attributed to reactivation due to infliximab was 3.4 times that of etanercept. However, the analysis also showed that both drugs caused a high proportion of new infections to progress directly to active disease. Ordinarily, more than 90% of new infections are contained by the immune response.



**Figure 3: Analysis of tuberculosis caused by TNF blockade by hidden Markov modelling and Monte Carlo simulation**

(A) Time to tuberculosis onset after starting infliximab or etanercept, as reported to the US Food and Drug Administration (solid lines) or as modelled (dotted line). (B) Analysis of 600 simulations, representing the range of reported tuberculosis case rates associated with infliximab or etanercept. Markov model parameters were identified by iterative methods that most closely reproduced observed time to onset distributions and target case rates. The median monthly rate of reactivation of latent *M tuberculosis* infection by infliximab based on this analysis was 12.1 times that of etanercept ( $p < 0.001$ ). Adapted with permission from reference 70. Copyright John Wiley & Sons Inc 2008.

A recent study in mice supports this conclusion. Plessner and colleagues<sup>71</sup> compared the effects of soluble murine TNFR2 fusion molecule to those of TNF antibody in mice acutely or chronically infected with *M tuberculosis*. The study found that lung colony-forming-unit counts increased and survival decreased in chronically infected mice (a model of reactivation) treated with TNF antibody, but not in those treated with TNFR2 fusion molecule. However, both TNF blockers increased bacterial burdens and mortality in acute *M tuberculosis* infection.

To summarise, these studies indicate that the anti-TNF antibodies infliximab and adalimumab pose a risk of tuberculosis several times greater than the soluble TNFR2 etanercept, and that this excess risk results from more efficient reactivation of latent *M tuberculosis* infection. By contrast, both classes of reagents share adverse effects on the outcome of newly acquired *M tuberculosis* infection.

### Structural and functional insights into differential infection risks

Several structural and functional differences among the TNF antagonists could account for the differences in granulomatous infection risk.

#### Pharmacokinetics

Substantial differences exist between infliximab, adalimumab, and etanercept with regard to their pharmacokinetics and dosing.<sup>33,34,43</sup> Peak blood levels of infliximab (80–100 µg/mL) are several times those of etanercept and adalimumab (5–10 µg/mL). It has been suggested that high peak levels of infliximab might account for its increased risk of infection.<sup>42,72</sup> However, the emerging experience with adalimumab indicates that it shares the increased tuberculosis risk of infliximab,<sup>64,65</sup> low blood levels notwithstanding.

#### Specificity

Infliximab and adalimumab bind only TNF, whereas etanercept can bind both TNF and lymphotoxin. However, lymphotoxin binding seems to be unrelated to infection risk, which is greater for the monoclonal antibodies. Preservation of lymphotoxin activity thus seems clinically insufficient to protect against tuberculosis, as has been shown experimentally in mice.<sup>18</sup>

#### sTNF binding kinetics

A study by Nesbitt and colleagues<sup>73</sup> used plasmon resonance to examine binding affinities of TNF blockers for sTNF. The equilibrium dissociation constants of etanercept, certolizumab, adalimumab, and infliximab were 33.4, 89.3, 157.4, and 227.2 pmol/L, respectively. Lower values indicate higher affinity, and potentially greater neutralisation. On the basis of this analysis, one would conclude that adalimumab and infliximab were less potent than etanercept and certolizumab. However, other studies indicate that TNF readily dissociates from etanercept, which releases more than 90% of bound cytokine after 2–3 h.<sup>74</sup> By contrast, dissociation of TNF from infliximab could not be detected in that study. Dissociation of etanercept from tissue TNF might account for the detection of only infliximab by immunohistochemical staining of involved lung in anti-TNF-treated chronically infected mice by Plessner and colleagues.<sup>71</sup>

#### sTNF binding stoichiometry

Etanercept binds only trimeric sTNF, at a ratio of one trimer per one etanercept dimer, precluding the formation of large antigen–antibody complexes. By contrast, infliximab and adalimumab readily bind both monomeric and trimeric sTNF. Since each monoclonal

antibody molecule contains two TNF binding sites, the potential exists for bridging interactions among multiple TNF trimers, resulting in the formation of large immune complexes.<sup>71,74–76</sup> This process could potentially lead to immune complex deposition, complement activation, and lupus-like phenomena such as glomerulonephritis and vasculitis. However, these phenomena have been reported as rare consequences of anti-TNF therapy with all agents.<sup>77,78</sup>

#### tmTNF binding

The affinities of TNF antagonists for tmTNF differ from those for sTNF, with etanercept binding less strongly than infliximab (1.15 nmol/L vs 0.45 nmol/L, respectively).<sup>74</sup> At equilibrium, four-fold more infliximab molecules bind to tmTNF compared with etanercept. In human lung carcinoma cells, certolizumab, infliximab, and adalimumab all neutralised tmTNF signalling with a potency two-fold greater than that of etanercept.<sup>73</sup> The dissociation of etanercept from tmTNF is similar to that of sTNF, being faster and more complete compared with infliximab.<sup>74</sup>

#### Cytotoxicity

Etanercept, infliximab, and adalimumab could all potentially mediate complement-dependent cytotoxicity and antibody-dependent cellular toxicity. Preliminary data suggest that when studied at high concentrations, all three TNF blockers induce complement-dependent cytotoxicity of tumour cells transfected to express high levels of tmTNF.<sup>79</sup> Both classes of TNF antagonists also have been reported to induce antibody-dependent cellular toxicity; limited data suggest that adalimumab and infliximab may be more potent inducers in this respect.<sup>79,80</sup> However, the clinical significance of these observations is uncertain, since one study that used clinically achievable concentrations of the three drugs in tuberculosis-stimulated whole blood cultures found no evidence of cytotoxicity.<sup>81</sup>

#### Apoptosis

Defective apoptosis in gut lymphocytes is thought to be central to Crohn's disease pathogenesis. Several studies have reported restoration of normal apoptosis in lamina propria T cells by TNF monoclonal antibodies in vitro (by use of terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling and other assays), and in vivo (by use of radiolabelled annexin V).<sup>82–87</sup> This caspase-dependent process occurs within hours to days of TNF antibody exposure. It requires three cytoplasmic serine tmTNF residues and results in upregulation of Bax, Bak, and p21 (WAF1/CIP1) proteins. Apoptosis caused by infliximab and adalimumab can be shown in tumour cells engineered to overexpress tmTNF.<sup>10</sup> Etanercept has no effect in this system, possibly because of weak tmTNF binding, or because the stoichiometry of binding (1:1) does not facilitate tmTNF cross-linking. The efficacy of certolizumab

in the treatment of Crohn's disease would seem to support the former hypothesis,<sup>38</sup> because its monovalent structure does not facilitate cross-linking (figure 1).

Induction of apoptosis other than in Crohn's disease T cells does not seem to be a major differentiating factor of the TNF antagonists.<sup>88–90</sup>

#### T-cell activation and cytokine expression

Several studies have reported inhibition of T-cell activation and cytokine expression (including interferon  $\gamma$ ) by TNF monoclonal antibodies in the absence of effects on viability.<sup>81,91–95</sup> In two studies, this was accompanied by inhibition of T-cell expression or proliferation of CD69 (an early activation marker).<sup>81,95</sup> Etanercept showed reduced or no effect in these systems. Antigen-induced production of interferon  $\gamma$  by T cells is required for host defences against *M tuberculosis*.<sup>19</sup> Mixed effects have been reported on interleukin 10, a regulatory cytokine associated with increased tuberculosis risk.<sup>81,86</sup>

Clinical trials and laboratory studies of certolizumab could help to determine the relative importance of each of the potential differentiating factors described in determining the tuberculosis risk of anti-TNF therapy. Because certolizumab lacks Fc, it cannot participate in complement activation or cell lysis.<sup>79</sup> Furthermore, its monovalent structure does not support immune complex formation or tmTNF cross-linking. However, it seems to act similarly to other TNF monoclonal antibodies with regard to binding to sTNF and tmTNF, and is effective as therapy for Crohn's disease.<sup>38</sup> Two phase III placebo-controlled studies of certolizumab in patients with rheumatoid arthritis (RAPID 1 and 2) have been reported in abstract form.<sup>39,40</sup> These trials were conducted internationally, including some sites in countries with increased tuberculosis incidence. Five active tuberculosis cases have been reported to date in each trial, yielding rates of 6.9 and 12.5 cases per 1000 patient-years of exposure. No cases of tuberculosis were reported in the control arms of these studies. Further analysis of these early reports to define the tuberculosis risk of certolizumab will likely provide additional perspective on the relation between structure and function of TNF blockers and their associated risk of granulomatous infection.

#### Diagnosis and treatment of latent tuberculosis infection

Our understanding of the human tuberculosis risks of the TNF blockers largely reflects experience garnered in regions of low tuberculosis transmission. In the USA, for example, both the prevalence of latent tuberculosis infection and the annual risk of acquiring infection in the general population are low.<sup>96,97</sup> As a result, most tuberculosis cases are thought to arise from reactivation of latent infection acquired in high prevalence regions. Current strategies to minimise tuberculosis risks in patients beginning anti-TNF therapy therefore emphasise detection and treatment of latent infection.

The greatest body of experience in the detection of latent tuberculosis infection relates to the tuberculin skin test (TST), which detects delayed-type hypersensitivity to purified protein derivative of *M tuberculosis* (PPD). Skin testing does not distinguish between persistent *M tuberculosis* infection and immunological memory of an infection that has been eradicated by chemotherapy or by a protective host response. Indeed, Markov modelling indicates that the proportion of patients with latent tuberculosis infection capable of reactivation due to TNF blockade is only a very small fraction of TST-positive rheumatoid patients.<sup>97</sup> The specificity of the TST as an indicator of latent tuberculosis infection is further reduced by the presence of antigens in PPD that are shared by non-tuberculous mycobacteria, including *M bovis* BCG. Vaccination with BCG is done in infancy in over 100 countries. BCG is not administered in the USA, mainly because of the concern that it interferes with TST. Indeed, a median reaction diameter of 16.5 mm was reported in previously skin-test-negative healthy US adults 1 month after vaccination.<sup>98</sup> However, this response was short lived, decreasing to 9.5 mm by 1 year. Reactions are smaller and decline more rapidly in infants. For example, a study of 1-year-old Ugandan children vaccinated at birth found that in the absence of exposure to an active tuberculosis case in the household, only 29% had TST responses greater than 5 mm.<sup>99</sup> Large diameter reactions in adults are therefore unlikely to result solely from BCG vaccination in infancy.

The specificity of testing for latent tuberculosis infection can be improved by the use of the antigens early secreted antigenic target 6 (ESAT6) and culture filtrate protein 10 (CFP10), encoded by genes absent from all BCG strains.<sup>100</sup> T-cell responses to these antigens are detected by in-vitro assays as the release of interferon  $\gamma$  (ie, interferon gamma release assays [IGRA]), either by ELISA (QuantiferON Gold, Cellestis, Carnegie, Victoria, Australia) or by ELISPOT (T.SPOT-TB, Oxford Immunotech, Oxford, UK). Some studies have reported reduced sensitivity of testing with ESAT6 and CFP10 compared with PPD,<sup>101</sup> although others have not.<sup>102</sup> Studies in India and Peru with the ESAT6 and CFP10 assays indicate that most TST reactivity in regions of high tuberculosis prevalence is indeed caused by *M tuberculosis* infection rather than merely BCG sensitisation.<sup>102,103</sup>

TST responses are reduced in rheumatoid patients.<sup>51,104,105</sup> TST sensitivity can be increased by reducing the threshold for positivity (5 mm) and by repeated testing (boosting) 7–10 days after a negative test. Both strategies increase test sensitivity at the cost of reduced specificity.<sup>106,107</sup> The sensitivity of screening can be further improved by chest radiography to identify regional scarring and hilar lymphadenopathy.

The effectiveness of such a strategy was assessed in Spain, through the BIOBADASER registry. Spain has moderately low tuberculosis prevalence (22 cases per 100 000 population) and low isoniazid resistance (4%).<sup>48,108</sup>

The registry was initiated in 2000, 1 year after infliximab became available. 32 cases of tuberculosis were reported among the 1648 patients treated with infliximab from 2000 to 2002,<sup>63</sup> a rate 23 times that in the general population. No cases occurred in 233 patients treated with etanercept.<sup>63</sup> Beginning in 2002, recommendations were put into place that required patients being considered for anti-TNF therapy to be screened by chest radiography and two-step TST using a 5 mm threshold. Patients with evidence of latent tuberculosis infection were to receive at least the first of 9 months' treatment with isoniazid before initiating anti-TNF therapy. Tuberculosis rates in infliximab-treated patients subsequently declined by 74%.<sup>63</sup> Isoniazid was well tolerated. Raised transaminase concentrations were reported in seven of the 324 patients treated with isoniazid for latent tuberculosis infection. No patients died or required admission to hospital because of liver failure.<sup>63</sup>

Large preventive therapy studies in the 1970s and 1980s established efficacy rates of 65% and 75% for isoniazid treatment durations of 24 and 52 weeks, respectively; a 12-week regimen was only 21% effective.<sup>109,110</sup> The Spanish experience therefore indicates that TNF blockade does not interfere with treatment of latent tuberculosis infection. Indeed, it is apparent from these data that preventing tuberculosis reactivation does not require the eradication of latent infection before starting TNF blockade, but merely that isoniazid treatment has been initiated. Current recommendations for 1 month of isoniazid before starting anti-TNF therapy may nonetheless be useful to ensure adequate isoniazid tolerability. The delay may be most appropriate for infliximab, given its shorter time to tuberculosis onset and higher tuberculosis risk.

In other settings, larger TST or IGRA responses have been linked to higher tuberculosis risk.<sup>111,112</sup> Skin testing without boosting, or using a 10 mm threshold, might be more appropriate for etanercept; however, this must be assessed by physicians on a case by case basis, since to date latent tuberculosis infection screening and treatment has only been shown to be of benefit for infliximab. Some patients must be switched from one TNF blocker to another during the course of therapy, owing to loss of response or tolerability. Patients being switched from etanercept to a TNF monoclonal antibody should be tested and treated for latent tuberculosis infection if they had not done so previously. Such testing might not be required for patients being switched to etanercept, since their period of highest risk of reactivation might have already passed.

The prevalence of isoniazid resistance varies greatly by country, with rates of 10–29% reported in parts of China and Russia.<sup>108</sup> Isoniazid treatment of latent tuberculosis infection in these regions will be less effective. Alternative regimens, such as rifampicin plus pyrazinamide, can be considered, although experience with these regimens has been limited because of reports of severe hepatotoxicity.<sup>113,114</sup>

Additional concerns arise because of the potential contribution of tuberculosis resulting from progression of new infection. The annual risk of tuberculosis infection is closely linked to tuberculosis prevalence; rates ten to 100 times higher than those in the USA have been reported in tuberculosis-endemic countries such as China and India.<sup>115,116</sup> Biological therapies are increasingly available in these regions despite incomplete safety profiles.<sup>117</sup> Progression of new *M tuberculosis* infection to disease will not be affected by treatments for latent infection, apart from, presumably, during the time the treatment is given. Tuberculosis cases appearing to be caused by failed treatment of latent infection in people from tuberculosis-endemic regions might instead represent progression of new infection occurring after cessation of isoniazid. In view of the high risks posed by both infliximab and etanercept for progression of new infection to active disease, alternative strategies for prevention might be required as the use of these drugs increases in tuberculosis-endemic regions.

### Management of incident tuberculosis cases

Many questions remain regarding optimum management of patients who develop tuberculosis as a consequence of TNF blockade. Most authorities recommend that anti-TNF therapy be halted in such cases until a response to antituberculosis therapy is evident, but clinical trials in this regard have not yet been done. A single case report describes the successful resumption of infliximab therapy after 2 months of tuberculosis treatment in a patient with peritoneal tuberculosis.<sup>118</sup> A small clinical trial of adjunctive etanercept in patients with newly diagnosed pulmonary tuberculosis reported trends toward accelerated microbiological and clinical responses in the etanercept arm.<sup>119</sup> A similar study of adjunctive high-dose methylprednisolone found that TNF levels in serum and cell culture were reduced by 60% compared with controls, and that sputum culture conversion to negative was hastened by 1 month compared with controls.<sup>120</sup> The accelerated microbiological response to these adjunctive treatments might be caused by superior penetration of tuberculosis drugs into granulomas, or to enhanced drug activity against metabolically active bacilli.<sup>121,122</sup> These studies indicate that anti-TNF therapy

does not hinder, and might facilitate, the response to standard tuberculosis therapy, but further research in this area is warranted.

Infliximab withdrawal in the setting of active tuberculosis might be associated with a paradoxical worsening of tuberculosis.<sup>123,124</sup> The clinical manifestations of such paradoxical reactions can include worsened fever, pulmonary infiltrates, hypoxia, lymphadenopathy, and the evolution of previously inapparent intracranial tuberculomas. Similar reactions have been described in tuberculosis patients with or without concomitant HIV infection, but paradoxical reactions associated with TNF monoclonal antibody withdrawal seem to be more severe. Disseminated tuberculosis has been the main clinical predisposing factor. The reactions seem to represent exaggerated recovery of otherwise normal antimicrobial immune responses. Paradoxical reactions must be differentiated from secondary superinfections (eg, aspergillosis), drug resistance, and true tuberculosis treatment failure. Some cases of paradoxical worsening as a result of infliximab withdrawal have been reported to require glucocorticoids, surgical excision, or resumption of anti-TNF therapy. One case has been reported in which infliximab was used to treat a severe, intractable paradoxical reaction involving the central nervous system in a patient with disseminated tuberculosis not previously on anti-TNF therapy.<sup>125</sup> Further research into the prevention and management of paradoxical reactions associated with the withdrawal of TNF blockade is needed.

### Conclusions

The introduction of TNF antagonists into clinical medicine has greatly advanced the treatment of several chronic inflammatory diseases, but has also resulted in a greater clinical awareness of the risks these therapies pose for granulomatous infections. Several studies have confirmed the increased risk of granulomatous infections of TNF antibodies compared with soluble receptor, particularly with regard to reactivation of latent *M tuberculosis* infection, which for infliximab occurs at a monthly rate 12 times that of etanercept. Differences in structure and function of these drugs seem to account for differences in their clinical spectrum of activity and their associated infection risks. Binding to tmTNF on activated T cells might be central to this process. Further clinical and laboratory studies are needed to better understand the relation between therapeutic effect, infection risk, and mechanism of action of these drugs, and to develop effective strategies for prevention and management of these infections.

#### Conflicts of interest

I have served as a consultant for Wyeth and Amgen, with fees totalling less than US\$10000 during the past 3 years. This Review was prepared while I was employed by PPD Inc, a company that undertakes research for the pharmaceutical and biotechnology industries and for governmental agencies. I am currently employed by Pfizer Global Research and Development. Neither company has any financial or other interest in any of the products or manufacturers mentioned in this Review.

#### Search strategy and selection criteria

Articles cited in this Review were obtained through searches of Medline, meeting abstract databases, and reference lists from key reviews. Search terms included the names of TNF antagonists with one or more of the following: "mechanism", "monocyte", "macrophage", "T cell", "cytokine", "lysis", "apoptosis", "granuloma", "granulomatous", "tuberculosis", "histoplasmosis", "coccidioidomycosis", or "aspergillosis". Priority was given to primary research publications. No date restrictions were placed on the searches.



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