

Reactivation of Latent Granulomatous Infections by Infliximab

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Although infliximab and etanercept share tumor necrosis factor (TNF) as a common therapeutic target, accumulating data indicate that infliximab (an anti-TNF monoclonal antibody) poses a greater risk of reactivation of latent granulomatous infections than does etanercept (a soluble TNF receptor). Similarly, infliximab is effective for the treatment of chronic granulomatous inflammatory conditions (e.g., Crohn disease) for which etanercept is ineffective. The ability of infliximab to disrupt established granulomas may be distinct from its ability to neutralize soluble TNF. Further research to elucidate the mechanism of the antigranuloma activity of infliximab is warranted.

TNF AND GRANULOMA BIOLOGY

Granulomas provide a strategy for containing intracellular infections that cannot otherwise be eradicated by host defenses. The ingestion by a lung macrophage of an indigestible pathogen, such as *Mycobacterium tuberculosis*, sets in motion a process in which neutrophils, macrophages, and, subsequently, cells with increasing specificity for *M. tuberculosis* (i.e., natural killer cells, $\gamma\delta$ cells, and CD4 and CD8 T cells) are recruited to the site of infection. Although these cells can express many potential antimicrobial mechanisms (or can facilitate their expression in infected macrophages), they are rarely successful in eradicating *M. tuberculosis* infection [1]. As a result, human mycobacterial immunity is primarily bacteriostatic and is associated with the ability of granulomas to serve as a physical barrier to mycobacterial dissemination. In humans, tuberculosis (TB) granulomas are composed of a central core of macrophages, multinucleated giant cells, and necrotic debris surrounded by macrophages and lymphocytes, which include CD4 and CD8 T cells and B cells [2]. Mycobacteria contained

within the interior of the lesion face a hostile microenvironment in which oxygen tension, pH, and micronutrients are all reduced. In response, bacilli undergo profound alterations in metabolism, biosynthesis, and replication [3]. The result is a semidormant state that is thought to be punctuated by occasional bursts of metabolic activity, as if the bacilli are “testing the waters” periodically to determine whether immunity has waned sufficiently to permit reactivation.

TNF is essential for the formation and maintenance of granulomas. The cytokine is expressed by macrophages and T cells in response to exogenous and endogenous signals, first as a cell-surface-associated form and then, after cleavage of membrane-anchoring domains, as soluble homotrimer [4, 5]. TNF stimulates the release of inflammatory cytokines, chemokines, and endothelial adhesion molecules, thus promoting the recruitment and activation of neutrophils and other cells and enhancing the microbicidal activity of macrophages and natural killer cells [6]. Animals that lack the gene for TNF or that are treated with neutralizing antibody fail to contain experimental infection with mycobacteria and other intracellular pathogens [7].

It is, therefore, not surprising that the use of anti-TNF therapies for the treatment of inflammatory conditions, such as rheumatoid arthritis (RA), has been associated with granulomatous infections. What was unanticipated, however, was that the 2 most widely used

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TNF blockers differ in both their therapeutic spectrum of activity and the risks they pose for reactivation of latent granulomatous infections.

TNF BLOCKADE AND GRANULOMATOUS INFECTIONS

In the late 1990s, two TNF blockers were introduced into clinical practice for the treatment of chronic inflammatory conditions. Infliximab (Remicade; Centocor) is a murine-human chimeric anti-TNF monoclonal antibody that binds diverse TNF moieties. It was approved by the US Food and Drug Administration (FDA) for the treatment of Crohn disease (CD) in 1998 and for the treatment of RA in 1999. Infliximab binds monomeric and trimeric TNF, both in solution and on cell surfaces. At present, >500,000 patients worldwide have been treated with infliximab [8].

Etanercept (Enbrel; Amgen) is a fusion of the extracellular portion of the p75 TNF receptor linked to the Fc domains of human IgG1. It was approved by the FDA for the treatment of RA in 1998, for the treatment of psoriatic arthritis in 2002, and for the treatment of psoriasis in 2004. Etanercept mainly binds soluble trimeric TNF. At present, >250,000 patients worldwide have been treated with etanercept [9].

The largest and most systematic studies of granulomatous infections in patients treated with these TNF blockers were recently published in *Clinical Infectious Diseases* [10, 11]. The studies were performed using the Adverse Event Reporting System (AERS), a voluntary reporting system established by the FDA for postmarketing surveillance of adverse drug events. Examination of >35,000 AERS reports from January 1998 through September 2002 identified 255 reports of granulomatous infections associated with infliximab use and 68 cases associated with etanercept use in the United States. The number of reports of granulomatous infections increased progressively during each year studied. The etanercept- and infliximab-treated patients described in these reports did not differ with respect to sex (60% vs. 59% were female, respectively) or concurrent methotrexate use (40% vs. 47%). However, etanercept-treated patients were younger (median age, 56 vs. 65 years; $P = .0125$, by log-rank test) and were more likely to have also received corticosteroids (62% vs. 37%; $P < .01$, by χ^2 test). Limited data, collected starting in 2002, indicated that, in 14% of cases, infliximab was used to treat CD, compared with no cases of in which etanercept was used to treat CD.

Centocor and Amgen had reported to the FDA that, as of September 2002, at total of 197,000 US patients had been treated with infliximab, and 113,000 had been treated with etanercept. On the basis of these data, we calculated that TB, histoplasmosis, coccidioidomycosis, and listeriosis all were reported with significantly greater frequency after treatment with infliximab, com-

pared with etanercept (table 1). A trend toward increased risk was apparent for nontuberculous mycobacteria. Only salmonellosis occurred with greater frequency after treatment with etanercept (2 cases vs. no cases after treatment with infliximab).

Several caveats are appropriate in the consideration of these findings. Because entries in the AERS database do not contain patients' identifying information and include only limited demographic data, we cannot be certain that the 2 populations of patients are comparable. We did find, for example, that etanercept-treated patients were younger, which possibly indicates a reduced risk of developing TB, but this phenomenon may have been counterbalanced by increased corticosteroid use, which is a recognized risk factor for reactivation of TB. RA itself may predispose patients to develop TB, although accurate data regarding this possibility are not available. It is not known whether CD has a similar effect. Cases may be reported to AERS by patients, physicians, or other health care providers; for this reason, duplicate reports may appear. Duplicate reports may also be filed as additional information regarding a case is collected. In those instances in which we could identify duplicate reports, the most recent (and most complete) report was selected. Reporting to any voluntary system may be incomplete. We attempted to minimize these potential biases by focusing on comparisons within the AERS database.

Approximately two-thirds of AERS reports included the time from initiation of anti-TNF therapy to onset of infection. Other immunocompromising conditions, such as AIDS, shorten the time to onset of diseases, such as TB, and increase overall risk. Unlike incidence data, time-to-onset data are not readily affected by bias because of incomplete reporting. The time to onset was significantly shorter in association with infliximab use than with etanercept use for TB and histoplasmosis and approached statistical significance for coccidioidomycosis. The cumulative proportion of cases of TB in relation to duration of anti-TNF therapy is shown in figure 1. Etanercept-associated cases of TB were distributed uniformly during the reporting period, with only 10% of cases occurring during the first 90 days of treatment. This random distribution suggests that these cases may have occurred as a result of the inability to control new *M. tuberculosis* infection. In contrast, 43% of infliximab-associated cases of TB occurred during the first 90 days of treatment, indicating that they likely represent reactivation of latent infection. This difference is consistent with an increased overall risk of TB due to infliximab use. Under the assumption that these patterns reflect the general distribution of time to onset of TB for patients receiving infliximab or etanercept, the annualized risk of developing TB during the first 90 days of treatment with infliximab was 95 cases/100,000 person-years, compared with 11 cases/100,000 person-years with etanercept. For comparison, the

Table 1. Absolute numbers and rates of granulomatous infections per 100,000 among US patients treated with infliximab or etanercept, as reported to the Food and Drug Administration Adverse Event Reporting System, from January 1998 through September 2002.

Infection	Infliximab (n = 197,000)	Etanercept (n = 113,000)	RR	P
Aspergillosis	17 (8.63)	7 (6.19)	1.39	.243
Candidiasis	20 (10.15)	6 (5.31)	1.91	.061
Bartonellosis	1 (0.51)	0 (0)563
Coccidioidomycosis	11 (5.58)	1 (0.88)	6.31	.013
Cryptococcosis	10 (5.08)	8 (7.08)	0.72	.179
Histoplasmosis	37 (18.78)	3 (2.65)	7.07	<.0001
Legionellosis	1 (0.51)	0 (0)563
Leprosy	1 (0.51)	0 (0)563
Listeriosis	17 (8.63)	1 (0.88)	9.75	.0006
Nontuberculous mycobacterioses	22 (11.17)	7 (6.19)	1.80	.066
Nocardiosis	7 (3.55)	1 (0.88)	4.02	.090
Pneumocystosis	1 (0.51)	0 (0)563
Salmonellosis	0 (0)	2 (1.77)	0.00	.031
Toxoplasmosis	4 (2.03)	0 (0)101
Tuberculosis	106 (53.81)	32 (28.32)	1.90	<.0001
Total	255 (129.44)	68 (60.18)	2.15	<.0001

NOTE. Data are no. of infections (no. of infections/100,000 treated patients), except where noted. *P* values were determined by use of Poisson analysis. RR, risk ratio (infliximab:etanercept). Data are from Wallis et al. [11].

annual incidence of TB in the United States during this period was 5 cases/100,000 person-years [12, 13].

This overall conclusion has been supported by a recent smaller study that examined the risk of coccidioidomycosis associated with the use of TNF blockers without relying on the AERS database [14]. The study found that the risk of developing coccidioidomycosis associated with the use of infliximab was significantly greater than that associated with the use of etanercept.

DIFFERENTIAL THERAPEUTIC EFFECTS OF TNF BLOCKERS

Differences between infliximab and etanercept have also emerged with respect to their therapeutic indications. Although they are equally effective for treatment of RA, only infliximab shows activity against granulomatous inflammatory diseases, such as Crohn colitis, sarcoidosis, or Wegener granulomatosis [15–18]. In patients with CD, infliximab can induce long-lasting remissions that are not readily explained by the pharmacokinetics of its binding to soluble TNF [19]. Together, these clinical observations indicate that infliximab has a capacity to disrupt established granulomas that is distinct from its ability to block soluble TNF.

Several hypotheses have been advanced to account for these observations. The dosing interval for infliximab is significantly longer than its half-life. The high levels of infliximab present

in the circulation immediately after dose administration may exert biological effects not present at lower concentrations. Infliximab binds diverse TNF moieties (monomeric and trimeric), including cell-associated forms; etanercept primarily binds soluble TNF, and only recognizes the active homotrimer. Infliximab also binds TNF with greater avidity, thus potentially neutralizing TNF more completely than does etanercept. However, infliximab lacks activity against lymphotoxin- α (TNF- β), which contributes to mycobacterial immunity and, presumably, is blocked by etanercept.

INDUCTION OF T CELL APOPTOSIS BY INFLIXIMAB

Recent studies have provided an alternate hypothesis: infliximab induces apoptosis memory T cells, whereas etanercept is antiapoptotic. Independent studies by van den Brande et al. [20] and di Sabatino et al. [21] indicate that, in CD, infiltrating effector memory T cells fail to undergo apoptosis under conditions that normally would induce cell death and that this defect is stably corrected by infliximab via caspase-dependent pathways. Memory CD4⁺ and CD8⁺ T cells comprise at least 2 functionally distinct subsets: central memory T cells, which home in on the T cell areas of secondary lymphoid organs, and effector memory T cells, which have acquired the capacity to migrate to nonlymphoid tissues and which preferentially ex-

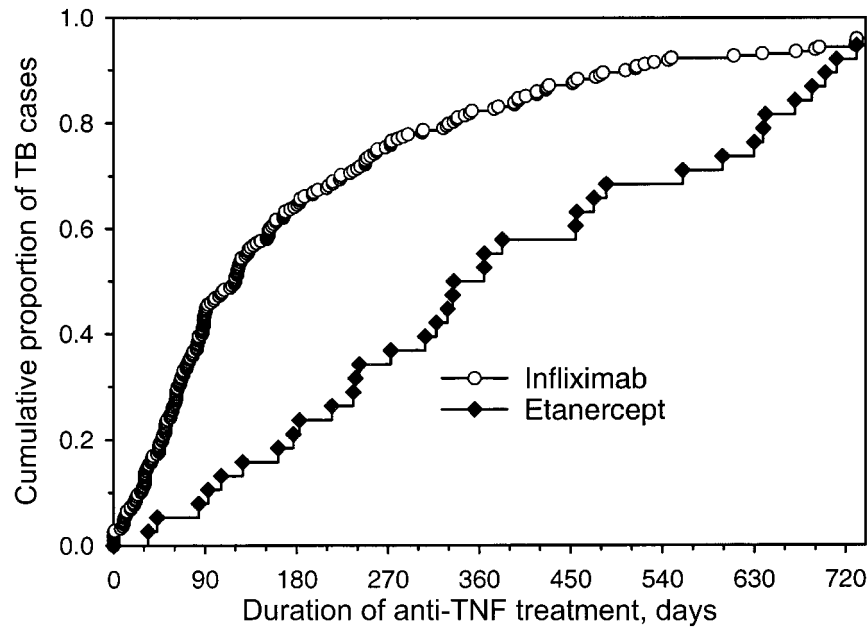


Figure 1. Cumulative proportion of tuberculosis (TB) cases in relation to the time of initiation of anti-TNF treatment. Each symbol denotes a case reported to the US Food and Drug Administration Adverse Event Reporting System from January 1998 through March 2003. The 2 curves differed by Kaplan-Meier analysis ($P = .00028$). The inflection in the curve at 90 days for infliximab is consistent with a shift in etiology at that time from re-activation of disease to progression of new infection. Data are from Wallis et al. [11].

press IFN- γ and TNF, rather than IL-2 [22, 23]. Normally, only a small fraction of effector memory cells become central memory T cells; instead, most die as a result of apoptosis. This strategy is essential to preserving long-term immune memory, because, in its absence, immune responses would be restricted overwhelmingly to recently encountered antigens. In CD, defective apoptosis appears to account for the accumulation of lymphocytes in the gut. Infliximab apparently corrects this defect by binding to T cell membrane-associated TNF. The effect is distinct from necrosis and is independent of complement but is otherwise unexplained, because the cytoplasmic tail of membrane TNF is not known to be involved in transmembrane signaling pathways and because TNF ordinarily provides an important signal for apoptosis of activated lymphocytes [24].

POSSIBLE INHIBITION OF APOPTOSIS BY ETANERCEPT

In contrast to CD, AIDS and TB are 2 conditions in which apoptosis is excessive, rather than defective, and in which apoptosis contributes to pathogenesis and accelerates disease progression [24–28]. TNF is also an important mediator of the local and systemic inflammatory response in TB and causes many of the clinical manifestations of the illness. In this context, we recently completed a phase 1 study of adjunctive etanercept during the initial 4 weeks of treatment of HIV-associated TB [29]. The main findings were that treatment with etanercept

resulted in an increase in CD4 T cell counts of 96 cells/ μ L, compared with an increase of 25 cells/ μ L in control subjects ($P = .1$), and that CD4 cell count and serum neopterin (a marker of macrophage activation [28]) were inversely correlated ($P < .01$). Similar increases in CD4 cell counts, attributed to inhibition of apoptosis, have been reported in 2 studies in which HIV-infected individuals were treated with prednisone [30, 31]. Further studies will be required to determine whether the increases we observed are due to inhibition of apoptosis and whether they are accompanied by other immunologic or clinical benefits.

SUMMARY

In summary, accumulating data indicate that infliximab poses a greater risk of reactivation of granulomatous infections than does etanercept, and this phenomenon reflects basic differences in the biological effects of these 2 drugs, with respect to survival of memory T cells. Additional research is required to determine how these differences can best be used to the advantage of patients with infectious and inflammatory conditions.

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