A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis

Robert S. Wallis[a,b], Peter Kyambadde[c], John L. Johnson[a], Libby Horter[a], Rodney Kittle[a,d], Monika Pohle[a], Constance Ducar[e], Monica Millard[a], Harriet Mayanja-Kizza[c], Christopher Whalen[a] and Alphonse Okwera[c]

Objective: Tumor necrosis factor (TNF), an important inflammatory mediator in tuberculosis, has been implicated in causing accelerated HIV disease progression in HIV-associated tuberculosis. However, TNF blockade, particularly by monoclonal antibody, has been associated with the reactivation of latent *Mycobacterium tuberculosis* infection by the impairment of mycobacterial immunity. This phase 1 study examined the safety, microbiology, immunology, and virology of TNF blockade using etanercept (soluble TNF receptor, Enbrel) during the initial treatment of HIV-associated tuberculosis.

Design: A single-arm trial, with key endpoints compared with historical controls, conducted in Mulago Hospital, Kampala, Uganda.

Subjects: Sixteen HIV-1-infected patients and 42 CD4-frequency-matched controls with sputum smear-positive tuberculosis and CD4 cell counts > 200 cells/μl.

Intervention: Etanercept 25 mg, eight doses administered subcutaneously twice weekly beginning on day 4 of tuberculosis therapy.

Main outcome measures: Serial examination, radiography, sputum culture, CD4 T-cell counts, plasma $\log_{10}$ HIV-RNA copy numbers.

Results: Trends towards superior responses to tuberculosis treatment were evident in etanercept-treated subjects in body mass, performance score, number of involved lung zones, cavitary closure, and time to sputum culture conversion. Etanercept treatment resulted in a 25% increase in CD4 cells by week 4 ($P = 0.1$ compared with controls). The change in CD4 cell count was inversely related to the change in serum neopterin, a marker of macrophage activation. There was no effect on plasma HIV RNA.

Conclusion: Etanercept can be safely administered during the initial treatment of pulmonary tuberculosis. Further studies are warranted to examine the effects of etanercept on T-cell numbers, activation and apoptosis in AIDS and tuberculosis.

AIDS 2004, **18**:257–264

Keywords: AIDS, clinical trial, etanercept, safety, soluble TNF receptor, tuberculosis, tumor necrosis factor

From the [a]Department of Medicine, Case Western Reserve University, Cleveland, OH, USA; [b]Department of Medicine, University of Medicine and Dentistry – New Jersey Medical School, Newark, NJ, USA; [c]Departments of Medicine and Microbiology, Makerere University, Kampala, Uganda; [d]Joint Clinical Research Center, Kampala, Uganda; [e]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Correspondence to Robert S. Wallis, Department of Medicine, UMDNJ-NJMS, 185 South Orange Avenue, MSB I-503, Newark, NJ 07103, USA.

Tel: +1 973 972 8778; fax: +1 973 972 8878; e-mail: r.wallis@umdnj.edu

Received: 6 February 2003; revised: 2 June 2003; accepted: 25 June 2003.

DOI: 10.1097/01.aids.0000104367.21567.d8

ISSN 0269-9370 © 2004 Lippincott Williams & Wilkins

Introduction

Tuberculosis is the most common presenting illness for AIDS patients worldwide. Despite appropriate tuberculosis treatment, such patients show an accelerated loss of CD4 T cells, and are at a greater risk of subsequent opportunistic infection and death than matched controls without tuberculosis [1,2]. These adverse clinical outcomes are accompanied by sustained cellular immune activation and increased rates of spontaneous and antigen-induced T-cell apoptosis [3–7]. Tumor necrosis factor (TNF), a cytokine abundantly produced by *Mycobacterium tuberculosis*-infected macrophages, has been implicated as a key mediator in this process, given its ability to promote both HIV expression and apoptosis [8,9]. TNF is also an important mediator of the local and systemic inflammatory response in tuberculosis, causing many of the clinical manifestations of the illness. However, clinical trials to examine the role of TNF in HIV-associated tuberculosis have to date been limited by the lack of potency and specificity of the possible interventions, which have included pentoxifylline, prednisolone, and thalidomide, drugs with diverse immunological and anti-inflammatory effects.

The present study examined the safety, microbiology, virology, and immunology of eight doses etanercept (Enbrel) given twice a week during the initial 4 weeks of treatment of HIV-1-infected patients with pulmonary tuberculosis. Etanercept is a recombinant protein consisting of the human type II (p75) TNF receptor coupled to the Fc portion of immunoglobulin G. Its binding of TNF and lymphotoxin (also known as TNF-β) has potent effects in rheumatoid arthritis and other chronic inflammatory conditions [10]. In this study, etanercept permitted the specific inhibition of TNF without otherwise disturbing a complex network of immune mediators.

TNF is essential for host defences against tuberculosis [8]. Patients treated with TNF inhibitors, particularly anti-TNF monoclonal antibody, are at increased risk of the reactivation of latent tuberculosis and other intracellular infections [11]. As the safety of etanercept in patients with active tuberculosis had not been established, this study was undertaken as a single-arm phase 1 trial with safety as its main objective. Its secondary objectives included immunological, virological, and microbiological endpoints, recognizing that the power of these observations would be limited by the small sample size. These endpoints were compared with CD4-matched control subjects selected from the placebo arm of a randomized trial of adjunctive prednisolone in HIV-1-associated tuberculosis, conducted at the same site during the preceding 2 years.

Methods

Subjects

Written informed consent and Institutional Review Board approval was obtained in accordance with US Department of Health and Human Services guidelines. Inclusion criteria required that subjects be HIV-1 seropositive with CD4 cell counts greater than 200 cells/μl, between 18 and 50 years of age, with an initial episode of pulmonary tuberculosis (based on a positive sputum acid-fast smear, chest radiography compatible with tuberculosis, and subsequent confirmation of at least one sputum specimen with growth of at least 10 colonies of *M. tuberculosis*).

Individuals were excluded if they had a previous history of treatment for tuberculosis; jaundice or abnormal liver enzymes [serum glutamic-oxaloacetic transaminase (aspartate aminotransferase) > 100 IU/l]; hemoglobin less than 8 g/dl; white blood cell count less than $3.0 \times 10^3/mm^3$; serum creatinine greater than 177 μM/l (2 mg/dl); Karnofsky performance scale score less than 50%; respiratory rate greater than 35/min; known active intravenous drug or alcohol abuse; treatment with any investigational agents, immunomodulators, chemotherapy or radiation therapy within 60 days; neoplasms other than basal cell carcinoma or in-situ carcinoma of the cervix; pregnancy or breast feeding; individuals requiring or likely to require corticosteroids; asthma; known infection with drug-resistant *M tuberculosis*; treatment with protease inhibitors during the preceding 6 weeks; change in antiretroviral therapy anticipated during the next 8 weeks; evidence of acute myocardial infarction, high-grade ventricular ectopy or other serious arrhythmias or conduction disturbance on screening electrocardiogram; history of multiple sclerosis; allergy or intolerance of trimethoprim or sulfonamides; or suspected meningeal or miliary tuberculosis. Individuals were not excluded for concurrent antiretroviral therapy other than protease inhibitors; however, no such treated subjects were screened.

Treatment

Enrolled subjects were admitted to the National TB Treatment Centre of Mulago Hospital, Kampala, Uganda. Subjects began standard short-course tuberculosis chemotherapy, consisting of 2 months of daily isoniazid, rifampin, ethambutol, and pyrazinamide, followed by 4 months of daily isoniazid and rifampin. The milligram daily doses of these four drugs were determined according to body weight: less than 50 kg, 300, 450, 800, 1500 mg; 50–70 kg, 300, 600, 1000, 2000 mg; greater than 70 kg, 300, 600, 1200, 2000 mg, respectively. Pyridoxine 50 mg was administered daily for 6 months. Etanercept 25 mg was administered subcutaneously twice a week for eight doses, starting on day 4 of tuberculosis therapy. Co-trimoxazole (160/

800 mg) was administered daily for one year, because of concerns about possible opportunistic infections as a result of etanercept, and because some studies found that this intervention improves survival in HIV-associated tuberculosis in Africa [12–14].

Week 2 safety evaluation

All subjects underwent a safety evaluation after the 4th dose of etanercept, in which changes from baseline to week 2 in plasma HIV RNA and quantitative sputum acid-fast microscopy were determined. These values, along with clinical data regarding signs and symptoms of HIV and tuberculosis, were reviewed by an external safety monitoring board. The discontinuation of etanercept treatment was recommended by the study protocol for subjects whose plasma HIV RNA increased by 1 log or more, or whose sputum acid-fast bacillus (AFB) counts increased by 0.2 logs or greater.

Outpatient tuberculosis treatment

Subjects were discharged to home after the completion of etanercept treatment at the end of week 4. The remainder of the tuberculosis therapy was self-administered. Adherence was monitored by attendance at scheduled monthly clinic appointments, dispensing records, and testing for urinary isonicotinic acid (Myco-Dyn Uritec; Symcon, Solana Beach, CA, USA) at monthly intervals. At each monthly clinic visit during tuberculosis treatment, a focused history and physical examination was conducted to identify signs and symptoms of tuberculosis and other potential HIV-related adverse events. The final examination was conducted 6 months after the completion of tuberculosis therapy.

Laboratory evaluations

Transaminase levels, creatinine, and complete blood counts were measured on weeks 4 and 8, and at the conclusion of tuberculosis treatment. Two sputum specimens were subjected to microscopic examinations and culture for mycobacteria each month during tuberculosis treatment, and 6 months after the completion of treatment. Sputum processing for quantitative microscopy, culture and drug susceptibility testing was performed as previously described [15,16]. Sputum culture conversion was defined as the first negative sputum culture without subsequent positive cultures. Plasma HIV RNA was measured by the HIV Amplicor Monitor test v1.5 manual method (Roche Molecular Systems, Indianapolis, IN, USA). Serum neopterin was measured by enzyme-linked immunosorbent assay (ICN, Costa Mesa, CA, USA).

Control subjects

Forty-two control subjects were randomly selected from among those recruited to the placebo arm of a randomized trial of adjunctive prednisolone in HIV-associated tuberculosis, conducted at the same clinical site from November 1998 to July 2000. A manuscript describing that study is currently in preparation. Its enrollment criteria and tuberculosis treatment were identical to those of the present study. CD4 T-cell counts and plasma HIV-RNA copy numbers of the control subjects were measured in the same laboratory as in the present study. The two protocols differed in that subjects enrolled in the prednisolone trial: (i) were not hospitalized for the first month of treatment; (ii) did not undergo a safety evaluation at week 2; and (iii) were not treated with co-trimoxazole for one year. Controls were randomly selected from three ranges of CD4 cell values so that the frequency distribution of their CD4 cell counts matched that of etanercept-treated subjects.

Statistics

Differences were examined by a two-tailed *t*-test, using SigmaStat (SPSS, Chicago, IL, USA), unless otherwise indicated. Paired *t*-tests were used to examine the change from baseline within subjects. The time to sputum culture conversion was analysed by survival analysis, using a log rank test.

Results

Subjects and controls

From August 2001 to April 2002, 16 subjects were enrolled from among 233 who were screened. A total of 196 were excluded because of negative HIV-1 serology, normal chest radiographs, negative sputum acid-fast microscopy, or the inability to produce sputum. Fifty-eight were excluded because of laboratory or clinical disease severity indicators outside the limits of the study. Three later withdrew consent or did not return for enrollment. Two subjects were eligible but were not enrolled because of a regulatory hold. Four subjects were excluded for other reasons. Some subjects had multiple reasons for exclusion. No subjects were excluded because of protease inhibitor use at the time of tuberculosis diagnosis; none initiated antiretroviral therapy during the period of tuberculosis treatment. The baseline characteristics of the subjects and 42 CD4-frequency-matched controls are shown in Table 1.

Discontinuations

Treatment with etanercept was initiated on day 4 of tuberculosis chemotherapy. All subjects underwent a safety evaluation on day 14, at which time changes from baseline in quantitative sputum acid-fast smear and plasma HIV-RNA copy numbers were reviewed by an external monitoring board. The mean $\log_{10}$ sputum AFB count was 6.65 ± 0.81/ml on entry, and 5.92 ± 0.75/ml on day 14 ($P = 0.009$). Two subjects showed more than a 0.2 log increase in sputum AFB

Table 1. Baseline characteristics of enrolled subjects and controls.

	Subjects		Controls
	Enrolled (n = 16)	As treated (n = 13)	(n = 42)
Sex (male)	10 (62%)	9 (69%)	26 (62%)
Age (years)	33.0 ± 8.0	34.2 ± 8.0	31 ± 7.1
BMI (kg/m^2)	20.3 ± 3.2	19.5 ± 3.3	19.4 ± 2.7
Laboratory results			
WBC (× 10^3/mm^3)	8.3 ± 2.4	8.2 ± 2.1	7.6 ± 2.3
Hemoglobin (gm/dl)	11.5 ± 1.4	11.6 ± 1.5	10.7 ± 1.6
Radiographic extent of disease			
Minimal	0 (0%)	0 (0%)	3 (6%)
Moderately advanced	4 (25%)	3 (23%)	10 (24%)
Far advanced	12 (75%)	10 (77%)	27 (64%)
Number of involved lung zones	4.4 ± 1.5	4.6 ± 1.2	3.7 ± 1.6
Cavitary disease	13 (81%)	11 (85%)	38 (90%)
Highest sputum AFB smear grade			
1+	0	0	4 (10%)
2+	0	0	2 (5%)
3+	16 (100%)	13 (100%)	36 (85%)
CD4 T cells (/μl)	394 ± 128	381 ± 126	407 ± 127
Plasma HIV RNA ($\log_{10}$ copies/μl)	5.00 ± 0.70	5.00 ± 0.70	4.85 ± 0.71

AFB, Acid-fast bacillus; BMI, body mass index; WBC, white blood cells.
Three subjects were discontinued from treatment at week 2.
Values indicate N (%) or mean ± SD.

counts during this interval; both were discontinued from etanercept treatment. In both instances, sputum AFB counts had been unusually low at baseline (4.35 and 5.28 $\log_{10}$ AFB/ml), indicating that these specimens may have been inadequate. Sputum cultures of both subjects converted appropriately to negative by week 8, and remained so subsequently.

The mean $\log_{10}$ plasma HIV-RNA copy number was 5.00 ± 0.7/ml on entry and 5.19 ± 0.9/ml on day 14 ($P = 0.3$). One subject had a greater than 1 log increase in copy numbers, and was discontinued from etanercept treatment. The clinical course of this subject is described in detail below.

Clinical evaluations

At the time of diagnosis, from 58 to 93% of subjects reported cough, dyspnea, inability to carry out usual daily activities, loss of appetite, fever, and episodes of sweating. Except for cough, these proportions declined progressively during treatment, being present in 20–60% of subjects by the end of week 2, and in less than 25% by the end of week 8. The weight at baseline was 53.2 ± 8.8 kg (mean ± SD). The weight gained by week 4 was 1.81 ± 1.8 kg in all subjects, and 2.18 ± 1.5 kg in those subjects who received all eight scheduled etanercept doses. The mean weight gain was greater than that observed in control subjects (1.73 ± 2.0 kg), although the difference was not statistically significant ($P = 0.40$). The mean increase in performance score in individuals whose baseline score was less than 90 was 8.6 ± 5.3 in all subjects, and 9.1 ± 5.4 in those subjects who received all eight scheduled doses. This value was greater than that observed in controls (5.1 ± 8.9), although the difference was not statistically significant ($P = 0.20$).

Sputum microbiology

Sputum cultures were positive for *M. tuberculosis* in four out of 14 (28.6%) at week 8; cultures of two subjects could not be evaluated because of contamination. The corresponding proportion in CD4-matched controls was also 28.6%. Overall, sputum culture conversion occurred slightly more rapidly in etanercept-treated subjects ($P = 0.05$), as indicated in Fig. 1. Seventy-seven tests for urinary isonicotinic acid were performed during the ambulatory phase of treatment (months 2–6); of these, only three were negative, indicating high compliance with treatment. All surviving subjects completed the final evaluation 6 months after the completion of tuberculosis therapy without relapse. Two of the 16 subjects had isoniazid-resistant isolates. Both showed satisfactory responses to treatment according to quantitative sputum microscopy on week 2, converted to negative sputum acid-fast culture by week 8, and remained so through their final evaluation.

Quantitative virology

As indicated in Table 2, log HIV-RNA copy numbers increased by +0.26 ± 0.4 copies/ml during etanercept treatment ($P = 0.043$). A corresponding increase of +0.05 ± 0.7 $\log_{10}$ copies/ml was observed during this interval in CD4-matched controls; the difference between etanercept-treated subjects and controls in changes from baseline was not significant ($P = 0.31$). At 6 months, HIV-RNA values in etanercept-treated subjects had returned to baseline (mean change −0.10, $P = 0.58$). Similarly, no change in HIV-RNA copy

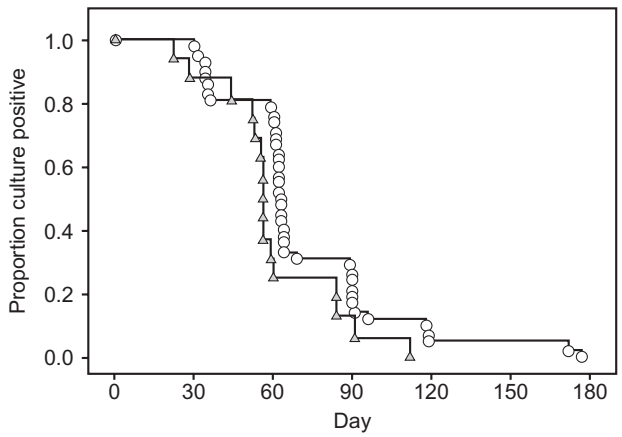

Fig. 1. Sputum culture conversion in etanercept-treated subjects and CD4-matched controls. Median (95% confidence interval) values for etanercept control subjects were 56 (55–57) and 63 (62–64) days, respectively ($P = 0.05$). Significance was determined by survival analysis, using a log rank test. ——○—— Control; ——△—— etanercept.

numbers was apparent in controls at month 4, the last evaluation in that study.

Immunology

The CD4 T-cell count on entry was 394 ± 128 cells/μl. Counts tended to increase by week 4 of etanercept treatment: the increase was 62 ± 195 cells/μl in all subjects ($P = 0.22$), and 96 ± 186 cells/μl in subjects receiving all eight scheduled etanercept doses ($P = 0.088$). Total lymphocyte counts tended to decrease during this interval (2770 ± 1500 cells/μl at baseline versus 2380 ± 700 cells/μl at week 4, $P = 0.3$). CD4 cell counts in matched controls increased by 24 ± 120 cells/μl ($P = 0.1$ compared with etanercept-treated subjects). The serum neopterin level at baseline was 10.5 ± 7.0 ng/ml. The change from baseline to week 4 was -0.4 ± 3.6 ng/ml in all subjects, and -0.7 ± 3.8 ng/ml in subjects receiving all eight etanercept doses. The corresponding change from baseline in controls was -0.1 ± 4.6 ng/ml ($P = 0.7$). An inverse relationship was observed between the change in neopterin and the change in the log CD4 cell count from baseline to week 4 using the Spearman rank test ($r = -0.37$, $P = 0.004$ in all subjects; $r = -0.50$, $P = 0.058$ in etanercept-treated subjects).

Chest radiography

Radiographs obtained during treatment were scored according to the number of lung zones involved. The change from baseline to month 6 tended to be superior in treated subjects compared with controls (-2.5 ± 1.1 versus -1.9 ± 1.5 lung zones, respectively), although this did not reach statistical significance ($P = 0.2$). Fifty-five per cent of etanercept recipients with cavitary disease at baseline had a closure of all cavities by month 6, versus 34% in CD4-matched control subjects ($P = 0.3$ by Fisher's exact test).

Adverse events

One subject, who entered the study with a CD4 cell count of 612 cells/μl, a $\log_{10}$ plasma HIV-RNA copy number of 4.18 copies/ml. and a serum neopterin level of 14.7 ng/ml, noted increased cough and dyspnea at week 2. The $\log_{10}$ plasma HIV-RNA copy number at that time had increased to 6.60 copies/ml. Levofloxacin and high-dose co-trimoxazole were added; etanercept was discontinued. Blood cultures and smears for malaria were negative. Bronchoscopy with bronchoalveolar lavage was non-diagnostic. The plasma HIV-RNA log copy number decreased to 5.17 on week 3, but increased again to 6.49 on week 4, at which time the CD4 cell count was 405 cells/μl. The subject developed unilateral leg edema during month 3 of treatment. Deep venous thrombosis, severe pulmonary hypertension, and presumed chronic pulmonary embolism were diagnosed at that time by Doppler ultrasound venography and echocardiography. The subject died despite anticoagulation. Autopsy was not performed. Sputum cultures obtained after 2 and 3 months of antituberculosis treatment were negative. A review of laboratory data and serial chest radiographs by an outside reviewer indicated a satisfactory response to tuberculosis therapy. The primary cause of death was thought to be pulmonary embolism.

Two subjects developed transient asymptomatic transaminase elevations after 1 and 2 months of treatment,

Table 2. Log_{10} plasma HIV-RNA copy numbers at baseline, and change from baseline during treatment, in all treated subjects, and in those receiving the full schedule of eight doses.

	All treated subjects		Full Rx (8 doses)		No Rx (controls)	
Timepoint	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Baseline	16	5.00 ± 0.7	13	4.96 ± 0.7	42	4.85 ± 0.7
Δ day 14	16	+0.19 ± 0.7	13	+0.02 ± 0.4		ND
Δ day 28	16	+0.33 ± 0.7	13	+0.26 ± 0.4	42	+0.05 ± 0.7
Δ month 4		ND		ND	42	+0.04 ± 0.6
Δ month 6	15	−0.11 ± 0.6	13	−0.10 ± 0.6		ND

ND, Not done; Rx, treatment.

thought to be caused by isoniazid. In both cases, isoniazid was re-instituted uneventfully once transaminase values had normalized. Two subjects developed dermatomal herpes zoster after 1 and 2 months of treatment, thought to be possibly related to etanercept. For comparison, one case of zoster occurred in the control subjects during the first 6 months of observation. Finally, one etanercept recipient was found to have intestinal perforation and peritonitis 11 months after study entry, thought to be caused by adhesions from previous abdominal surgery.

Discussion

Many questions remain regarding the mechanisms leading to T-cell loss in AIDS. Recent studies have implicated activation-induced apoptosis of uninfected 'bystander' T cells as a major factor, rather than direct viral cytopathicity or CD8-mediated cytotoxicity [17,18]. TNF provides an important signal for the apoptosis of activated lymphocytes [9]. The observation that TNF blockade in HIV-associated tuberculosis results in increased numbers of circulating CD4 cells supports the potential role of this cytokine in HIV–tuberculosis co-pathogenesis. The inverse relationship between the CD4 cell count and serum neopterin, a marker of macrophage activation, is consistent with this observation [19]. Similar increases in CD4 cell counts, attributed to the inhibition of apoptosis, have been reported in two studies in which HIV-infected individuals were treated with prednisone [20,21]. Further studies will be required to determine whether the increases observed in this study are caused by the inhibition of apoptosis, whether they are accompanied by other immunological or clinical benefits, and whether they occur in subjects with lower baseline CD4 cell counts.

The inhibition of TNF as a therapeutic modality is not without potential hazard. Animals lacking the gene for TNF or its receptors, or treated with neutralizing antibody or soluble TNF receptor, show increased susceptibility to *Mycobacterium bovis* bacillus Calmette–Guerin, *M. tuberculosis*, Listeria, Cryptococcus, and other intracellular pathogens [8,22–24]. Patients with rheumatoid arthritis or inflammatory bowel disease treated with neutralizing monoclonal antibody to TNF are at increased risk of the reactivation of latent *M. tuberculosis* infection [25]. Nonetheless, the current study demonstrates that treatment with the TNF inhibitor etanercept does not interfere with the response to treatment for pulmonary tuberculosis, as assessed by serial clinical examination, chest radiography, sputum microscopy and culture. Indeed, several treatment-related parameters in the present study (weight gain, performance score, sputum culture conversion, radiographic extent of disease and cavity closure) showed trends towards superiority in the etanercept group. Sputum culture status after 8 weeks of treatment is an indicator of the risk of relapse [26,27]. The lack of a deleterious effect on this parameter is therefore significant. In this respect, etanercept is similar to corticosteroids and pentoxifylline, which modulate the inflammatory response in tuberculosis but do not interfere with tuberculosis treatment [28–31]. Larger, randomized placebo-controlled trials will be required to determine whether etanercept improves the response to tuberculosis therapy by accelerating sputum culture conversion.

Within the limits imposed by the small sample size and short treatment interval, etanercept treatment also appeared to be safe with respect to other potential HIV-related adverse events. The single fatality occurred in a subject whose elevated serum neopterin at baseline placed him at a greater than fourfold increased risk of death [5]. Although deep venous thrombosis and pulmonary embolism have been reported in controlled clinical trials of etanercept in patients with rheumatoid or psoriatic arthritis, the incidence of these adverse events has not been related to the administered dose, nor has it differed significantly from that in control subjects. The incidence of deep venous thrombosis in tuberculosis has been estimated to be 3–10%, apparently reflecting hemostatic changes induced by the acute inflammatory response [32–34]. Further studies of the impact of HIV-1 infection on venous thrombosis in tuberculosis are warranted.

Etanercept treatment did not result in a reduction of plasma HIV RNA, despite the recognized importance of TNF as a signal for HIV expression via nuclear factor B, and the apparent role of the inflammatory response in tuberculosis driving local HIV expression [35–38]. This negative finding may indicate that factors other than TNF regulate HIV expression in tuberculosis.

In summary, adjunctive treatment with the TNF inhibitor etanercept did not interfere with the response to treatment for pulmonary tuberculosis in 16 HIV-1-infected individuals, in whom CD4 T-cell counts increased by 25%. Further studies are warranted to examine the effects of etanercept on T-cell activation and apoptosis, and to determine whether its addition to standard tuberculosis therapy confers any clinical benefits.

Acknowledgements

The authors would like to thank the members of the external safety review board, Allison Elliott, Jeurgen Freers, Moses Kamya, Peter Katahaa, Peter Mugyenyi, and Pontiano Kaleebu, as well as Margaret Wyza, Roy

Mugerwa, David Hom, and Thomas Daniel for their timely assistance with this study. They would also like to thank Sr Clare Drajoru and the nursing staff of the National TB Treatment Centre, Wards 5 and 6, Mulago Hospital, for providing outstanding nursing care during the inpatient phase of the study.

Sponsorship: Financial support was provided by a grant from Immunex Corporation (Seattle, WA, USA), and by National Institutes of Health contract NO1-AI45244/ AI95383.

References

1. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. **Accelerated course of human immunodeficiency virus infection after tuberculosis.** *Am J Respir Crit Care Med* 1995, **151**:129–135.
2. Whalen CC, Nsubuga P, Okwera A, Johnson JL, Hom DL, Michael NL, *et al.* **Impact of pulmonary tuberculosis on survival of HIV-infected adults: a prospective epidemiologic study in Uganda.** *AIDS* 2000, **14**:1219–1228.
3. Hirsch CS, Toossi Z, Othieno C, Johnson JL, Schwander SK, Robertson S, *et al.* **Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy.** *J Infect Dis* 1999, **180**: 2069–2073.
4. Wallis RS, Vjecha M, Amir Tahmasseb M, Okwera A, Byekwaso F, Nyole S, *et al.* **Influence of tuberculosis on human immunodeficiency virus (HIV-1): enhanced cytokine expression and elevated beta 2-microglobulin in HIV-1-associated tuberculosis.** *J Infect Dis* 1993, **167**:43–48.
5. Wallis RS, Helfand MS, Whalen C, Johnson JL, Mugerwa RD, Vjecha M, *et al.* **Immune activation, allergic drug toxicity, and mortality in HIV-positive tuberculosis.** *Tubercl Lung Dis* 1996, **77**:516–523.
6. Hirsch CS, Toossi Z, Vanham G, Johnson JL, Peters P, Okwera A, *et al.* **Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis.** *J Infect Dis* 1999, **179**:945–953.
7. Mayanja-Kizza H, Johnson JL, Hirsch CS, Peters P, Surewicz K, Wu M, *et al.* **Macrophage-activating cytokines in human immunodeficiency virus type 1-infected and -uninfected patients with pulmonary tuberculosis.** *J Infect Dis* 2001, **183**:1805–1809.
8. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. **The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection.** *Cell* 1989, **56**:731–740.
9. Oliveira Pinto LM, Garcia S, Lecoeur H, Rapp C, Gougeon ML. **Increased sensitivity of T lymphocytes to tumor necrosis factor receptor 1 (TNFR1)- and TNFR2-mediated apoptosis in HIV infection: relation to expression of Bcl-2 and active caspase-8 and caspase-3.** *Blood* 2002, **99**:1666–1675.
10. Husni ME, Maier AL, Mease PJ, Overman SS, Fraser P, Gravallese EM, *et al.* **Etanercept in the treatment of adult patients with Still's disease.** *Arthritis Rheum* 2002, **46**:1171–1176.
11. Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, *et al.* **Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management.** *Lancet Infect Dis* 2003, **3**:148–155.
12. Badri M, Ehrlich R, Wood R, Maartens G. **Initiating co-trimoxazole prophylaxis in HIV-infected patients in Africa: an evaluation of the provisional WHO/UNAIDS recommendations.** *AIDS* 2001, **15**:1143–1148.
13. Maynart M, Lievre L, Sow PS, Kony S, Gueye NF, Bassene E, *et al.* **Primary prevention with cotrimoxazole for HIV-1-infected adults: results of the pilot study in Dakar, Senegal.** *J Acquired Immune Defic Syndr* 2001, **26**:130–136.
14. Anglaret X, Chene G, Attia A, Toure S, Lafont S, Combe P, *et al.* **Early chemoprophylaxis with trimethoprim–sulphamethoxazole for HIV-1-infected adults in Abidjan, Cote d'Ivoire: a randomised trial. Cotrimo-CI Study Group.** *Lancet* 1999, **353**: 1463–1468.
15. Wallis RS, Perkins M, Phillips M, Joloba M, Demchuk B, Namale A, *et al.* **Induction of the antigen 85 complex of *M. tuberculosis* in sputum: a determinant of outcome in pulmonary tuberculosis.** *J Infect Dis* 1998, **178**:1115–1121.
16. Siddiqi SH, Hwangbo CC, Silcox V, Good RC, Snider DEJ, Middlebrook G. **Rapid radiometric methods to detect and differentiate *Mycobacterium tuberculosis*/*M. bovis* from other mycobacterial species.** *Am Rev Respir Dis* 1984, **130**:634–640.
17. Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE. **CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause?** *Nat Med* 2002, **8**:319–323.
18. Kovacs JA, Lempicki RA, Sidorov IA, Adelsberger JW, Herpin B, Metcalf JA, *et al.* **Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV.** *J Exp Med* 2001, **194**:1731–1741.
19. Schoedon G, Troppmair J, Fontana A, Huber C, Curtius HC, Niederwieser A. **Biosynthesis and metabolism of pterins in peripheral blood mononuclear cells and leukemia lines of man and mouse.** *Eur J Biochem* 1987, **166**:303–310.
20. Andrieu JM, Lu W, Levy R. **Sustained increases in CD4 cell counts in asymptomatic human immunodeficiency virus type 1-seropositive patients treated with prednisolone for 1 year.** *J Infect Dis* 1995, **171**:523–530.
21. Wallis RS, Kalayjian R, Jacobson JM, Fox L, Purdue L, Shikuma CM, *et al.* **A study of the immunology, virology, and safety of prednisone in HIV-1-infected subjects with CD4 cell counts of 200–700/mm.** *J Acquired Immune Defic Syndr* 2003, **32**: 281–286.
22. Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ. **TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection.** *J Immunol* 2002, **168**:4620–4627.
23. Yamada K, Yoshino K, Sekikawa K, Madarame H, Yagita H, Nakane A. **Effect of a matrix metalloproteinase inhibitor on host resistance against *Listeria monocytogenes* infection.** *FEMS Immunol Med Microbiol* 2000, **29**:187–194.
24. Kaneko H, Yamada H, Mizuno S, Udagawa T, Kazumi Y, Sekikawa K, *et al.* **Role of tumor necrosis factor-alpha in *Mycobacterium*-induced granuloma formation in tumor necrosis factor-alpha-deficient mice.** *Lab Invest* 1999, **79**:379–386.
25. Cush JJ, Matteson EL, American College of Rheumatology. *FDA advisory committee reviews safety of TNF inhibitors*. Available at: http://www.rheumatology.org/research/hotline/0901tnf.html. Accessed September 2001.
26. Mitchison DA. **Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months [Letter].** *Am Rev Respir Dis* 1993, **147**:1062–1063.
27. Benator D, Bhattacharya M, Bozeman L, Burman W, Cantazaro A, Chaisson R, *et al.* **Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial.** *Lancet* 2002, **360**:528–534.
28. Nemir RL, Cardona J, Vaziri F, Toledo R. **Prednisone as an adjunct in the chemotherapy of lymph node-bronchial tuberculosis in childhood: a double-blind study. II. Further term observation.** *Am Rev Respir Dis* 1967, **95**:402–410.
29. Tripathy SP, Ramakrishnan CV, Nazareth O, Parthasarathy R, Santha Devi T, Arumainayagam DC, *et al.* **Study of chemotherapy regimens of 5 and 7 months' duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in South India.** *Tubercle* 1983, **64**: 73–91.
30. Wallis RS, Nsubuga P, Okwera A, Whalen C, Mugerwa RD, Oette D, *et al.* **Pentoxifylline in human immunodeficiency virus-seropositive tuberculosis: a randomized, controlled trial.** *J Infect Dis* 1996, **174**:727–733.
31. Wallis RS, Johnson JL, Okwera A, Nsubuga P, Whalen CC, Mugerwa RD, *et al.* **Pentoxifylline in human immunodeficiency virus-positive tuberculosis: safety at 4 years [Letter].** *J Infect Dis* 1998, **178**:1861.
32. White NW. **Venous thrombosis and rifampicin.** *Lancet* 1989, **2**:434–435.
33. Robson SC, White NW, Aronson I, Woollgar R, Goodman H, Jacobs P. **Acute-phase response and the hypercoagulable state in pulmonary tuberculosis.** *Br J Haematol* 1996, **93**: 943–949.
34. Turken O, Kunter E, Sezer M, Solmazgul E, Cerrahoglu K,

Bozkanat E, *et al*. **Hemostatic changes in active pulmonary tuberculosis.** *Int J Tubercl Lung Dis* 2002, **6**:927–932.
35. Osborn L, Kunkel S, Nabel GJ. **Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B.** *Proc Natl Acad Sci U S A* 1989, **86**:2336–2340.
36. Toossi Z, Mayanja-Kizza H, Hirsch CS, Edmonds KL, Spahlinger T, Hom DL, *et al*. **Impact of tuberculosis (TB) on HIV-1 activity in dually infected patients.** *Clin Exp Immunol* 2001, **123**:233–238.
37. Mayanja-Kizza H, Wajja A, Wu M, Peters P, Nalugwa G, Mubiru F, *et al*. **Activation of beta-chemokines and CCR5 in persons infected with human immunodeficiency virus type 1 and tuberculosis.** *J Infect Dis* 2001, **183**:1801–1804.
38. Collins KR, Quinones-Mateu ME, Wu M, Luzze H, Johnson JL, Hirsch C, *et al*. **Human immunodeficiency virus type 1 (HIV-1) quasispecies at the sites of *Mycobacterium tuberculosis* infection contribute to systemic HIV-1 heterogeneity.** *J Virol* 2002, **76**:1697–1706.