Experimental therapies for sepsis directed against tumour necrosis factor

Robert C. Read*

Department of Molecular and Genetic Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK

Tumour necrosis factor (TNF) has been identified as an important mediator involved in the generation of sepsis syndrome. Two major strategies have evolved for counteracting the effects of TNF in patients with severe manifestations of sepsis: neutralization by anti-TNF antibodies and competitive antagonism of TNF with synthetic soluble TNF receptors. Clinical trials with murine monoclonal antibodies against TNF have shown that this agent is able to reduce early morbidity and mortality, but with no reduction in 28 day mortality. A clinical study with a synthetic 75 kDa soluble TNF receptor failed to show any benefit with this drug and indeed there was higher mortality at higher doses. Trials of a 55 kDa soluble TNF receptor are continuing and this drug is apparently safe. Drugs that modify TNF in vivo may be a useful component of future management of sepsis, either as monotherapy or as part of a combined strategy of immunomodulation.

Introduction

A number of observations suggest that tumour necrosis factor (TNF) is a central mediator of sepsis. Endotoxin injection results in a predictable release of TNF from macrophages and neutrophils into the circulation.\(^1\) Administration of TNF to patients with cancer results in characteristic features of sepsis including hypotension, consumption of clotting factors and dysfunction of major organ systems.\(^2,3\) Examination of patients with sepsis reveals that TNF can be detected over several days,\(^4\) falling concentrations correlate with survival,\(^5\) and high concentrations correlate closely with severity of disease.\(^6\) Polymorphism of the TNF-\(\alpha\) promoter region has been shown to influence outcome in malaria\(^7\) and in patients with sepsis.\(^8\) However, some reservation should be attached to the concept that TNF is the dominant cytokine responsible for the generation of sepsis syndrome because concentrations detected in sepsis vary enormously,\(^9\) and TNF is but one component of a complex system of pro- and anti-inflammatory mediators.

The host response to secreted TNF is mediated by 55 kDa and 75 kDa surface receptors. Transmembrane components of these receptors are shed into the circulation spontaneously. They have been demonstrated to block the bio-availability of circulating TNF\(^10,11\) and are detectable during sepsis.\(^12\) Together with other anti-inflammatory cytokines, e.g. interleukin 4 (IL-4), IL-10, and cytokine antagonists, e.g. circulating IL-1 receptor antagonist (IL-1ra), they presumably form part of the host’s natural negative feedback during the inflammatory response to infection.

Two major strategies for neutralizing circulating TNF have been investigated: (i) blockade of circulating TNF by monoclonal antibodies and (ii) competitive antagonism of TNF with synthetic soluble TNF receptors.

Anti-TNF antibodies

Early studies with monoclonal antibodies and polyclonal sera were shown to reduce mortality in animal models of sepsis. Dose-dependent protection was demonstrated in experimental challenge with endotoxin,\(^13\) Escherichia coli,\(^14,15\) Pseudomonas aeruginosa\(^16\) and Staphylococcus aureus.\(^17\) Although these studies did not mirror the therapeutic situation in that maximal responses were achieved when anti-TNF antibody was administered either before or at the same time as bacterial challenge, the observed effects were sufficiently encouraging to lead to the use of experimental anti-TNF in clinical trials.

Clinical studies

In early phase II trials a murine monoclonal anti-TNF antibody (CB 0006) was well tolerated by patients with severe

*Tel: +44-(0)114-272-4072; Fax: +44-(0)114-273-9926; E-mail: r.c.read@sheffield.ac.uk

© 1998 The British Society for Antimicrobial Chemotherapy
sepsis, and although no overall benefit was demonstrated, there was a trend towards improved outcome in those patients with the highest TNF levels at entry, who were treated with anti-TNF antibody at a dose of 10 μg/kg. In more recent clinical studies, highly purified murine IgG1 subclass monoclonal antibodies have been used after calculation of appropriate doses from neutralizing studies in eight different animal species. An early study of the anti-TNF-α monoclonal antibody BAY X 1351 in 20 uninfected patients and 16 septic patients demonstrated that doses up to 15 mg/kg were safe and that the monoclonal antibody had a serum half-life of 50–54 h. A though anti-murine antibodies were produced by study patients, no manifestations of hypersensitivity resulted.

Two major trials employing the same murine monoclonal antibody have now been published. In the first study (NORSEPT), 994 North American patients with sepsis syndrome were enrolled and prospectively stratified into those who were in shock (defined by a sustained decrease in systolic blood pressure to <90 mmHg or a decrease of 40 mmHg from baseline for at least 30 min, that was refractory to an intravenous volume challenge of at least 500 mL of normal saline), or non-shocked. Patients were randomized to receive a single infusion either of placebo or anti-TNF at a dose of either 7.5 mg/kg or 15 mg/kg within 12 h of a sepsis syndrome diagnosis being made. An interim analysis of the first 800 patients enrolled revealed that, amongst non-shocked patients, there was no evidence of efficacy and, indeed, a slightly higher mortality amongst those receiving anti-TNF-α compared with placebo. Enrolment of non-shocked patients was, therefore, discontinued and the study was continued for a further 5 months. A total of 478 patients with shock were ultimately evaluated. Amongst these patients there was a high incidence of sepsis refractory to an intravenous volume challenge of at least 500 mL of normal saline and 44 of 160 (28%) patients in the placebo group and 36% in patients receiving MAK 195F, mortality was not significantly different between the three groups (placebo, 42.9%; 3 mg/kg, 36.7%; 15 mg/kg, 44.6%), nor was any effect on 3 day mortality observed, in contrast to the NORSEPT study. A nalysis of secondary endpoints suggested some modification of the natural history of the disease. Among those patients who survived, the duration of shock was 3.7, 3.9 and 7.0 days for the 15 mg/kg, 3 mg/kg and placebo groups, respectively. Life table analysis indicated a significantly more rapid reversal of shock with both doses. It was also shown that the mean number of patients with multi-organ failure decreased by administration of anti-TNF antibody.

Recent studies with repeated administration of the F(ab')2 fragment of a monoclonal anti-TNF antibody, MAK 195F, have also shown no overall benefit in 28 day mortality. However, though the study was considerably smaller than NORSEPT and INTERSEPT, studies with this antibody have suggested that patients with higher initial IL-6 concentrations may experience greater benefit with the drug. In patients presenting with baseline IL-6 concentrations of >1000 pg/mL mortality was 80% in the placebo group and 36% in patients receiving MAK 195F, 10 μg/kg at 8 h intervals over 3 days.

Taken together, these studies failed to demonstrate a significant effect of anti-TNF-α monoclonal antibody upon 28 day all-cause mortality, although it did significantly modify the natural history of disease to some extent. A major problem for the conduct of these studies has been the heterogenous nature of the patients studied, with a high incidence of chronic underlying diseases. In such patients, the use of 28-day mortality as a primary endpoint might fail to discriminate between active treatment and placebo in the context of severe underlying disease. A useful model of sepsis in a relatively homogenous population has been provided by the Jarisch-Herxheimer reaction in patients with Borrelia recurrentis infection. Following antibiotic treatment of this condition, patients predictably experience fever, rigors and hypotension accompanied by a rise in serum TNF, IL-1 and IL-8 concentrations. A ministration of a sheep polyclonal anti-TNF antibody prior to antibiotic therapy significantly reduced clinical and cytokine responses to antibiotic therapy compared with use of a placebo.
Soluble TNF receptor (sTNFR)

There are two major types of TNF-α receptors of mol. wt 75 kDa and 55 kDa which exist in surface-bound and circulating forms. A recombinant human TNF receptor construct, p75 sTNFR: IgG1 (p75), composed of two molecules of the extracellular portion of the 75 kDa TNF receptor covalently linked to the hinge region of human immunoglobulin IgG1, has been developed. This chimeric construct is designed to prolong the circulating half-life of sTNFR, which is very short. A additionally, because TNF exists as a trimer in solution, a fusion protein consisting of two sTNFR sites increases the affinity of the construct for TNF compared with monomeric sTNFR. Hypothetically, Fc receptor-bound sTNFR should act as a matrix for removal of TNF-α from the circulation. In a murine model of E. coli sepsis, p75 reduced peak TNF-α levels and early mortality, but failed to prevent overall mortality over the entire period of the study.

One clinical study with p75 has been disappointing. In this study 141 patients were randomized to receive placebo or p75 at doses of 0.15, 0.45 or 1.5 mg/kg as a single infusion over 30 min. In placebo and low dose recipients, 28 day mortality was equivalent (30%). The higher doses were associated with significantly increased mortality (0.45 mg/kg, 48%; 1.5 mg/kg, 53%). The result of this trial was somewhat alarming, particularly as p75 has been shown to be safe and possibly beneficial in patients with rheumatoid arthritis. It is possible that the harmful effects of p75 resulted from retention of p75-TNF-α complexes within plasma, with consequent slow release of TNF-α into the circulation and disturbance of ‘normal’ homeostatic mechanisms. Alternatively, if TNF-α activity is totally prevented by adsorption with higher doses of p75, a loss of the protective effects of the antagonized immune response may have been responsible for the increased mortality.

A animal model of Gram-negative sepsis suggested that the 55 kDa soluble TNF receptor construct p55 sTNFR: Ig1 (p55) may be superior to p75 in terms of a protective efficacy. In this model (in which p75 delayed but did not prevent death), animals given p75 had persisting circulating TNF detected by ELISA. The p55 chimeric construct abolished detectable TNF activity and markedly reduced mortality compared with placebo. Although both p55 and p75 bind TNF-α with similar high equilibrium binding constants they have different kinetics of binding and release, which occurs approximately 100-fold faster with p75 than p55, thus p55 may be pharmacokinetically superior. Results from a 44 centre, phase II clinical trial of p55 sTNFR have now been reported and this revealed no effect on 28 day mortality of p55 at lower doses of 0.008 mg/kg and 0.042 mg/kg but a trend towards decreased 28 day mortality at the highest dose (0.083 mg/kg) with a 36% reduction compared with placebo ($P = 0.07$). At this dose there was also a trend towards reduction in organ dysfunction. The study drug was apparently safe (in contrast to p75). When patients were stratified according to IL-6 concentrations, no increased benefit was seen amongst patients with the highest concentration of IL-6, in contrast to the MAK 195 (anti-TNF-α) study.

Combination immunotherapy

In addition to TNF, IL-1 has been implicated as one of the pro-inflammatory cytokines that may mediate septic shock, and has been shown to act synergistically with TNF in production of deleterious effects. In animal models, inhibition of IL-1 has been protective. The hypothesis that combined inhibition of IL-1 and TNF might be more productive than inhibition of each alone has been tested in an animal model of endotoxaemia. The combination of recombinant IL-1ra and type 1 sTNFR did not provide additional protection over and above that provided by IL-1ra and sTNFR alone when study drugs were administered before or shortly after the endotoxin injection, but, in those animals in whom treatment was delayed until 7 h after the initial endotoxin insult, combination therapy was shown to be protective, in contrast to monotherapy, which was not. This benefit was not confirmed in a neutropenic rat model of P. aeruginosa sepsis; despite a significant protective effect of either IL-1 receptor antagonist or sTNFR used alone, combination therapy was uniformly lethal, a finding which suggests that a combination of these two agents should not be administered to humans.

Discussion

The results of studies using experimental therapies that reduce TNF concentrations have been disappointing, with the reservation that monoclonal anti-TNF antibody clearly modifies the disease to some extent. There are two possible reasons for these results; either the central hypothesis (that TNF is the central mediator of sepsis and its inhibition can reverse the disease process) is incorrect, or the studies performed have been flawed in design or size. The central hypothesis may be incorrect; TNF is an important pro-inflammatory mediator, but is only one of a network of pro-inflammatory molecules that include IL-1 and IL-6 and, furthermore, anti-inflammatory agents including IL-4, IL-10, IL-11, IL-13, sTNFR, IL-1Ra antagonists and transforming growth factor-β are released to control inflammation and restore homeostasis. The interrelationship between these agents is, as yet, undetermined, and will vary between patients, and in differing circumstances. The finding of at least some significant modification of the disease process in both large clinical trials of monoclonal anti-TNF antibody and the Jarisch–Herxheimer model, and the phase II study of p55 lends support for the assertion that TNF is an important mediator of sepsis. Regarding the size and design of clinical trials, both of the large anti-TNF antibody trials conducted so far were carefully...
monitored by evaluation committees. In both studies, patient groups were well matched in terms of severity of disease and background chronic disease. However, in view of the heterogenous nature of the disease it is possible that potential benefits in terms of mortality were diluted. There may be subsets of shocked patients who might benefit from the use of the study drugs but who cannot be currently discriminated because of the use of 28 day mortality as the primary endpoint in an exceptionally sick group of patients. Of great interest in this regard is the finding of possible efficacy of anti-TNF antibody therapy in patients with elevated IL-6 levels, though this has not been confirmed in other studies. It may be that exclusion of patients with chronic underlying conditions would be appropriate in trials of these potentially useful therapies in future.

There is general consensus that further investigation of these agents is warranted, but this will benefit from a better understanding of the pathogenesis of this disease and an appropriate choice of subjects for study.

References


