Inhibition of tumour necrosis factor alpha in idiopathic membranous nephropathy: a pilot study

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Abstract

Background. Tumour necrosis alpha has been implicated in the pathogenesis of autoimmune disorders was to evaluate the safety, tolerability and potential efficacy of the tumour necrosis factor alpha (TNF-α) inhibitor, etanercept (ET), in patients with idiopathic membranous nephropathy (MN).

Methods. Patients with biopsy-proven MN, nephrotic-range proteinuria and clearance of creatinine 50 ml/min or more were included in the study. Exclusion criteria were treatment with steroids or cyclosporine during the previous 3 months, or cytotoxic agents within 6 months prior to entry. ET was administered subcutaneously, 25 mg twice per week for 3 months. Plasma levels of TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), soluble intercellular adhesion molecule type 1 (sICAM-1), E-selectin, and the soluble form of tumour necrosis factor receptor-55 (sTNFR-55) were measured on entry and at Months 3, 6, and 9 after commencing therapy.

Results. Twelve patients were entered in the study (four females/eight males, mean time from diagnosis 8.3 months). The therapy was well tolerated; no infections or other adverse events were recorded by the end of follow-up. Two patients exhibited complete remission of proteinuria for at least 4 years. No significant change was found in the levels of TNF-α, IL-1, IL-6, IL-8 and IL-10 during the study. Similarly the levels of E-selectin and sICAM-1 were not significantly altered by therapy. Although we found no change in sTNFR-55 at 3 and 6 months, the levels of sTNFR-55 were found significantly decreased 9 months after therapy (mean difference from baseline: 334 ± 527 pg/ml, P = 0.028).

Conclusion. Short-term use of ET in a small series of patients reduced sTNFR-55 levels but did not exhibit any significant clinical effect in the majority of patients.

Keywords: etanercept; membranous nephropathy; tumour necrosis factor alpha

Introduction

Idiopathic membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome in Caucasian adults, with a highly variable clinical course and prognosis [1]. A recent report implicates anti-neutral endopeptidase antibodies in its pathogenesis. These were found in a pregnant woman and transferred to her fetus, who developed a severe form of membranous glomerulonephritis prenatally [2,3]. Although the primary mechanism in the pathogenesis of proteinuria in MN [4] is considered to be the deposition of immunoglobulins along with components of the complement system on the epithelial side of the glomerular basement membrane, a contributory role of cellular immunity is also implied by several studies [5,6]. This role is supported by evidence of increased expression of tumour necrosis factor alpha (TNF-α) in the glomeruli [5], high urinary levels and activation of the complement cascade together with and without certain TNF gene polymorphisms [11,12] in the affected patients [6–10]. In the era of molecular biology, experimental evidence is available supporting the concept of a TNF-dependent cytokine cascade and uncovering this cytokine as a potential therapeutic target [13].

TNF-α is a major pro-inflammatory cytokine produced in response to various stimuli [14,15] not only by monocytes and macrophages but also by glomerular and mesangial cells [16]. Circumstantial data suggest that it may serve as an important autocrine and paracrine factor in glomerular injury [17]. It exists in soluble and transmembrane forms with diverse biological effects [18], mediated by two distinct receptors: TNFR1 (55 kDa; p55) and TNFR2 (75 kDa; p75), present in the majority of cells [19].

Its effect on mesangial [17] and glomerular epithelial cells [20] and on the secretion of several mediators [21] is of interest, particularly in nephrotic syndrome, where
the epithelial cell damage is the key pathological finding. Accordingly, in vitro data suggest that TNF-α may have cytotoxic activity, which affects cell adhesion to human endothelial cells [22] via NF-κB activation and expression of ICAM-1 and E-selectin [23].

Etanercept (ET) is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kDa (p75) TNF receptor [24], used in the treatment of rheumatoid arthritis [25,26]. In light of the putative role of TNF in MN [10] and experimental data suggesting a potential positive effect from its inhibition [11,12], we examined whether ET may be of benefit in patients with MN. Likewise, in various immune-mediated diseases, including rheumatoid arthritis [13,27,28], Crohn’s enteritis [29] and ankylosing spondylitis [30], the efficacy of anti-TNF-α inhibiting treatment is well established. Also in some forms of vasculitis, such as Takayasu arteritis and Behcet’s disease [31], inhibition of TNF-α has been proved to be of benefit. To this end, we conducted a phase I–II study to evaluate its safety and initial efficacy in idiopathic MN.

Patients–methods

Study design and criteria for response and withdrawing

This was a pilot study, to assess the safety and efficacy of ET in patients with idiopathic MN. Patients were recruited between January 2001 and December 2003. Partial remission was defined as a >50% decrease in proteinuria and absolute proteinuria <3 g/day while complete remission was defined as a >50% decrease in proteinuria and an absolute protein excretion <0.3 g/day. Response to therapy was defined as a reduction in proteinuria allowing any certain patient to achieve partial or complete remission that remained for at least 6 months. Relapse was defined as an increase in 24-h urine protein excretion by the same rates. Criteria for withdrawing included pregnancy, serious infection or other medical emergency, persistent (more than 2 weeks) increase in serum creatinine by 50% or more and poor compliance.

Selection of patients

Patients were eligible if they had all of the following prerequisites: (a) age older than 18 years; (b) a diagnosis of MN proven by kidney biopsy within the previous 2 years; (c) proteinuria of at least 3 g/day; (d) minimum clearance of creatinine 50 ml/min, estimated using the Cockcroft–Gault formula; and (e) a normal chest x-ray and negative PPD within 3 months before entry. Any patient with normal chest x-ray but positive PPD received therapy for latent tuberculosis for 6 months before entering the study. All patients signed an informed consent, and the protocol was approved by the IRB. Patients were initially excluded if tested positive for hepatitis B virus, hepatitis C virus and human immunodeficiency virus or had other acute/chronic active infection. Additionally, we excluded patients with uncontrolled diabetes mellitus, women with MN who were pregnant or in the nursing phase, as well as patients with demyelinating neurological disease or haematological disorders. All patients with a history of exposure to cytotoxic agents, such as chlorambucil or cyclophosphamide, within 6 months before consideration for entry to this study, and/or use of cyclosporine or steroids within 3 months were also excluded.

Baseline evaluation

All patients had a full baseline physical examination prior to entry, including body weight and blood pressure measurement. Laboratory parameters were also measured including (a) markers of renal function: 24-h protein excretion (the mean of three consecutive urine collections, serum creatinine, estimated glomerular filtration rate) and (b) haematology/chemistry profile: counts of erythrocytes, white blood cell counts, platelets, lipid profile, albumin.

Treatment protocol

ET was administrated subcutaneously in a dose of 25 mg twice per week for 3 consecutive months with the option for those patients responding to be treated for 3 additional months. All patients were followed for a minimum of 18 months after cessation of therapy.

Concomitant therapy

Medications that were allowed to be given as symptomatic treatment included (a) non-steroid anti-inflammatory agents only occasionally, in low doses as pain relievers; (b) angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs) if these had already been used before entry in the study but no further adjustments of the dose were done after the initiation of ET therapy; and (c) warfarin, heparin and diuretics. No steroids were allowed during the study.

Evaluation of patients during follow-up

All participants were evaluated on a monthly basis with physical examination and laboratory tests including renal and liver function indexes, haematological profile, urine analysis and with 24-h urine collections for proteinuria. Moreover, the patients were tested every 3 months for serum lipid profile, ANA, anti-dsDNA antibodies if needed, complement levels, RF, plasma levels for TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10), E-selectin, s-ICAM-1 and sTNFR-55.

Collection of samples and cytokine measurement of biomarkers

All samples were collected in sterile 10-ml tubes containing EDTA and 50 μl of aprotinin, immediately cooled and promptly centrifuged. The separated plasma was stored at –80°C until measurement. Serum levels of sICAM, E-selectin and soluble TNFRI were measured using commercially available enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA, cat #BBE1B, BBE2B and DRT100, respectively) according to the manufacturer’s instructions. The minimum detectable concentrations of s-ICAM-1, E-selectin and soluble TNFRI (pg/ml) were 0.57 ng/ml, 1.69 ng/ml and 7.8 pg/ml, respectively.

Statistical analysis

The median, mean values and standard errors of means for cytokines and immunoglobulins were calculated at time points 0, 3 months, 6 months and 9 months after treatment. To detect changes in the levels of these parameters, Wilcoxon signed-rank tests for related samples according to baseline values were performed. The P-values of <0.05 were considered as being statistically significant. Analysis was performed using SPSS 13.0.

Results

Study population characteristics

A total of 12 patients were entered between January 2001 and December 2003. Clinical and demographic characteristics of the included patients are shown in Table 1. A renal biopsy had revealed stage II of MN in eight patients, stage I in three patients and stage III in one patient. The patients had disease with a mean duration of 8.3 months. One patient had been treated with chlorambucil and cyclosporine, 2 and 1 year before entry, respectively; both treatments were shown ineffective.
Safety and tolerability

All patients tolerated the treatment with ET well; no infection or other side effect was observed during the treatment phase or the follow-up period. Two patients developed ANA (previously negative) in low titres, but they did not exhibit any symptoms or signs consistent with systemic lupus erythematosus.

Clinical outcomes

After a 3-month therapy with ET, we recorded complete sustained remission in 2 out of 12 patients (16.6%) (Figure 1) while the rest showed no significant change in proteinuria. Therapy with ET was continued for 3 additional months in the responders and was discontinued in the non-responders as planned per protocol. The two patients who achieved remission remained in remission after a total follow-up of 4 years. The mean serum creatinine was not changed significantly during the study, but one patient showed an increase from 1.4 mg/dl (at baseline) to 2.6 mg/dl. A complete workup including repeat of kidney biopsy revealed acute renal failure attributed to acute tubular necrosis itself due to hypovolaemia resulting from diuretics abuse; the patient recovered renal function following modification of diuretic therapy.

Serum levels of cytokines and adhesion molecule levels

The mean value of serum TNF-α levels prior to the institution of therapy (baseline) was 44 ± 91 pg/ml. The mean value of serum IL-6 was 3.1 ± 1.6 pg/ml at baseline with all the patients being within the normal range. The mean values of serum levels for IL-10 and IL-1 were lower than the detection range of the assay used. No significant changes in the serum levels of TNF-α, IL-1, IL-6, interleukin-8 (IL-8) and IL-10 were detected (Wilcoxon-signed rank test). Similarly, serum levels of E-selectin and sICAM-1 did not significantly change after treatment with ET.

Although no significant alteration was observed in the serum sTNFR-55 levels 3, and 6 months post-therapy, a significant decrease was identified 9 months post-therapy, when compared with the corresponding values at baseline (mean difference from baseline: 333.8 ± 526.5 pg/ml, P = 0.028, Wilcoxon-signed rank test) (Figure 2). A borderline significant increase in serum IgG1 levels 6 months post-therapy compared to baseline was observed (P = 0.018). Accordingly, serum IgG2 levels were increased 6 and 9 months post-therapy when compared with the values at baseline (P = 0.028 and P = 0.046, respectively) (data not shown).

Discussion

This is the first pilot clinical trial demonstrating clinical outcomes and laboratory measurements after a 3-month administration of ET, a proven selective anti-TNF-α agent, in patients with idiopathic MN. All patients showed a good profile, with respect to safety and tolerability, although ET was not proven efficacious in reducing proteinuria in

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**Table 1. Demographic and clinical characteristics of patients who received etanercept**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>53 (19–69)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>8/4</td>
</tr>
<tr>
<td>Ethnicity (Caucasian/other)</td>
<td>12/0</td>
</tr>
<tr>
<td>Time from diagnosis (months), median (range)</td>
<td>1 (0–24)</td>
</tr>
<tr>
<td>Renal biopsy stage</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>5</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>1</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>1</td>
</tr>
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<td>Serum creatinine (mg/dl), mean (SEM)</td>
<td>1.1 (0.1)</td>
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<tr>
<td>GFR (ml/min), mean (SEM)</td>
<td>93.1 (6.8)</td>
</tr>
<tr>
<td>Proteinuria (g/day), mean (SEM)</td>
<td>9.4 (1.5)</td>
</tr>
<tr>
<td>Serum albumin (g/dl), mean (SEM)</td>
<td>2.9 (0.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Effect of etanercept treatment in proteinuria (A), serum albumin concentrations (B) and serum creatinine (C) in patients with idiopathic MN. At 3 months, we recorded complete sustained remission in 2 out of 12 patients while the rest had no significant change in proteinuria. Therapy with ET was continued in the responders for 3 additional months while in the rest of the patients it was discontinued per protocol. These two patients are still in remission after a total follow-up of at least 4 years.
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Fig. 2. Changes in soluble ICAM (A), E-selectin (B) and soluble TNF-receptor (C) following treatment with etanercept in patients with idiopathic MN. Serum levels of these molecules were determined by ELISA at the indicated time points. The asterisk (*) denotes the statistically significant difference from baseline (t = 0) values.

this small series of patients under the specific dose and scheme.

In view of data showing that TNF-α is an important mediator in MN [11,12], we thought that its inhibition might be of benefit in these patients. In addition to genetic polymorphisms of the TNF-α gene in patients with MN [11,12], TNF-α is found in the cytoplasm of glomerular epithelial cells [32] and specifically in human MN [5]. Serum TNF-α levels were increased in patients with MN [32,33], and other renal diseases [34]. These findings support the notion that TNF-α may play a pathogenic role in the induction or maintenance of glomerular barrier dysfunction in humans. Aiming to explore this possibility we employed ET, a TNF-α antagonist with very good pharmacokinetic properties [35] based on data from healthy volunteers and rheumatoid arthritis patients.

However, several factors differentiate MN from any other clinical entity and might be involved in the absence of efficacy in the present trial. For instance, the level of inhibition of TNF-α provided by the specific therapeutic agent used in this trial might not be adequate to block the disease process under the particular circumstances of nephrotic syndrome. Since the pharmacokinetics of these agents is largely not known in cases with significant proteinuria, the most appropriate dose and scheme can only be hypothesized. As a result, it is possible that a more prolonged or more intensive course of therapy with ET might be efficacious.

A diversity of results of such therapy has been reported across various autoimmune diseases. However, the distinctive features characterizing the pathogenetic mechanism implicated in each case as well as the involvement of additional organs and/or systems might be responsible. For instance, the role of TNF-α was studied in the accelerated model of anti-GBM glomerulonephritis [36] and attenuation was reported after its inhibition, while in the heterogeneous rat model of anti-GBM glomerulonephritis, treatment with a TNF-α antagonist was shown to prevent completely the development of crescents [37]. Whether the disease process itself and/or the resulting type of glomerular lesion are involved in the cycle of the TNF-α function is of question.

Yet, TNF blockade is also feasible with the chimeric anti-TNF-α antibody, Infliximab, and Adalimumab, a fully human monoclonal anti-TNF antibody [38]. Both Infliximab and Adalimumab bind to TNF-α preventing it from activating its receptors. Nevertheless, both of them have been shown to result in a range of beneficial effects among patients with autoimmune disorders [13,27,28,29,30,39]. For instance, the efficacy of ET and Infliximab in patients with rheumatoid arthritis seems to be comparable [40] and in patients with Crohn’s enteritis, ET seems not to be effective [41]; the third agent Adalimumab has been shown to be helpful in both.

Accordingly, most of the human studies which explore the bio-inhibition of TNF-α in ANCA-associated vasculitis have not conferred encouraging results [42], response also following [43] a substantial rate of adverse events, including infections, thrombotic events and a case of lymphoma was recorded while relapses were frequent. Data on the use of ET are even less promising since the only double-blind, placebo-controlled, multi-centre trial showed no significant differences in remission rates, disease activity or damage between those who received ET and the placebo group [44]. Remarkably, a significant number of cancer events were recorded solely in the ET group during the follow-up time.

As demonstrated by studies using inhibition of TNF-α in autoimmune diseases, differences in the working mechanism of each medication might explain the diversity of effects. Specifically, Infliximab by binding soluble and membrane-bound TNF-α has an increased capacity to inhibit TNF-α-mediated cytotoxicity and TNF-α-induced endothelial cell activation [45]. Furthermore, Infliximab, but not ET, can induce an anti-inflammatory response by reverse signalling through membrane bound TNF-α [46] while these agents have
different effects on T lymphocytes [47,48] and monocytes [49].

Another issue that might interfere with the success of therapy in MN is that the actual activity of the disease is unlikely to be assessed in any certain patient. Currently, we can only describe the clinical but not the immunological status of the disease using parameters such as proteinuria or the degree of renal impairment. Potential indicators, to show if the involved process is active or not, would allow the clinician to decide if any immunomodulating intervention might be futile.

In our study, the levels of serum TNF-α were not altered significantly by ET therapy, but we recorded a reduction of s-TNFR levels 9 months after initiation of therapy, which possibly reflects a decline in the activity of the TNF-α system. To date, no experiments studying the pharmacokinetics of ET in nephrotic patients with primary glomerulonephritis have been reported [50–53]. Nonetheless, enhanced expression of TNF-α may reflect a secondary result of monocyte activation, rather than a primary cellular defect in glomerulonephritis [32,36,37]. This possibility is supported by findings of increased plasma and/or urine levels in other renal disorders [33].

Additional circumstantial data obtained with the use of pentoxifylline (PTX) support a role for TNF in glomerular disease. PTX, best known for its use in vascular disease, when used in nephrotic patients due to diabetes mellitus or MN led to reduction of proteinuria [54,10]. The drug has strong anti-inflammatory properties, and reduces the production of cytokines in normal and disease states [55]. This agent also regulates the expression of adhesion molecules, such as ICAM-1, thereby affecting the binding of leukocytes to endothelium [56,57]. PTX suppressed TNF-α production by monocytes/macrophages [58], and reduced ICAM-1 expression by mononuclear cells and proximal renal cells [59,60]. In experimental crescentic glomerulonephritis, mRNA for TNF-α mRNA and ICAM-1 [61,62] were significantly suppressed by PTX. PTX exhibits a non-selective type of modulatory effect on synthesis and secretion of TNF-α [55,56]; yet, the anti-proteinuric result of PTX might be attributed to its rheological actions, by increasing the deformability of erythrocytes and reducing blood viscosity, glomerular hydraulic pressure and consequently proteinuria [63]. Indeed PTX is an antagonist of adenosine, and blockade of adenosine receptors may reduce hyperfiltration and proteinuria [64,65]. Therefore, PTX has been considered for the treatment of chronic kidney disease independently of the primary cause [67,68].

Despite that both receptors of TNF-α are involved in the regulation of ICAM-1 expression [46] and several adhesion molecules to endothelium [56,57], in our study, ICAM-1 and E-selectin levels were not altered by therapy with ET. We also failed to record changes in serum; Suranyi et al. showed that therapy with cyclosporine failed to reduce the levels of TNF-α in nephrotic patients, which were significantly higher than those in the healthy group [33]. We measured higher baseline plasma TNF-α levels compared with the ones reported in his study [33]. Steroids that their patients had received before measurement may have influenced baseline TNF-α levels [66] as, in our study, we selected patients who had not been treated with any kind of immunomodulation before entry.

Of interest, two functional polymorphisms of the TNF-α gene are strongly associated with the occurrence of MN [12]. Increased levels of TNF-α in the plasma and urine, and excessive expression of the TNF-α gene in the kidneys of patients with MN [69] might be linked with these polymorphisms. Whether there are carriers of such mutations of the TNF-α gene, within the MN population, who might benefit from a certain type, level or duration of inhibition of the TNF-α remains to be seen. Accordingly, the genetic profile of each patient with rheumatoid arthritis has been shown to interfere with the response to immunomodulating agents while the effect of ET therapy was significantly associated with a specific genetic variation [70]. In this context, resolution of nephrotic-range proteinuria after a 6-month treatment with ET in two cases with another disorder caused by a mutation in TNFR1 is noteworthy [71]. Nevertheless, the use of Infliximab led to decreased proteinuria in a small number of patients with lupus nephritis when added to background therapy with methotrexate or azathioprine.

In conclusion, a 3-month therapy with ET in a series of 12 patients with idiopathic MN was shown to be safe and well tolerated. The activity of the TNF-α system was probably reduced as this was reflected in the serum sTNFR-55 levels, but no significant therapeutic benefit or alteration of markers of the activation of the endothelium was observed. Besides, the lack of a control group does not allow us to rule out the possibility of spontaneous remission in two remitted patients. However, these data reiterate the importance of TNF-α in the pathogenesis of glomerular injury. Whether a longer course with ET or utilization of another inhibitor of TNF-α can be clinically effective remains to be elucidated by further investigation.

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