Could anti-inflammatory cytokine therapy improve poor treatment outcomes in dialysis patients?

Iain C. Macdougall

Department of Renal Medicine, King’s College Hospital, London, UK

Abstract
Mortality in dialysis patients is greater than that in the general population across all age groups. The disparity in mortality is greatest among patients aged under 35 years. Chronic kidney disease (CKD) is associated with the malnutrition, inflammation and atherosclerosis (MIA) syndrome, which helps to explain the high mortality rates among patients with CKD. Paradoxically, CKD patients exhibit signs of immune suppression as well as immune system activation. Chronic inflammation and immune system activation are not only integral to the MIA syndrome, but also may underlie resistance to erythropoietin treatment in patients with anaemia. Chronic immune system activation is reflected by abnormally raised T-lymphocyte and monocyte expression of both pro- and anti-inflammatory cytokines. Patients who respond well to erythropoietin treatment exhibit fairly normal expression of these cytokines. Patients who persistently fail to respond, however, express abnormally raised levels of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), which are also known to inhibit erythropoiesis. Paradoxically, these patients also express abnormally high levels of the anti-inflammatory cytokines interleukin (IL)-10 and IL-13. Although anti-inflammatory in nature, these cytokines might also affect erythropoiesis. One strategy to overcome the problem of chronic inflammation in anaemic patients with CKD may be treatment with the phosphodiesterase inhibitor, pentoxifylline. Preliminary results suggest that once-daily treatment with 400 mg of pentoxifylline orally not only can reduce T-cell expression of TNF-α and IFN-γ, but can also restore the response to erythropoietin and improve haemoglobin levels. Ongoing studies will investigate further the use of pentoxifylline in erythropoietin resistance.

Keywords: anaemia; anti-inflammatory cytokine therapy; cytokines; erythropoietin resistance; pentoxifylline

Introduction
Annual mortality in the age group under 35 years is almost 1000 times greater in patients undergoing dialysis than in the general population [1]. The difference is reduced in older age groups as mortality in the general population increases with age, although mortality rates remain higher in patients receiving dialysis in all age groups. It is highly likely that the high mortality rates among patients with chronic kidney disease (CKD), in particular the high rates of cardiovascular mortality, are related to the inflammatory nature of uraemia, which has been linked to the high prevalence of malnutrition and atherosclerosis in patients with CKD—the malnutrition, inflammation and atherosclerosis (MIA) syndrome [2]. The underlying chronic inflammatory state is characterized by enhanced immune system activation, which is evident even in the early, pre-dialysis, stages of CKD (Table 1).

Paradoxically, uraemic patients are both immunosuppressed, which predisposes them to infections, and in a state of chronic immune activation. Immune activation involves both the T lymphocytes (T cells) and monocytes. In uraemic patients, the T cells, depending on their type, produce a range of pro-inflammatory and anti-inflammatory cytokines. CD8⁺ T cells produce the pro-inflammatory cytokines interleukin-2 (IL-2) and tumour necrosis factor-α (TNF-α), while CD4⁺ cells produce the pro-inflammatory cytokine interferon-γ (IFN-γ) (type 1 helper T-cells, Th1) together with the anti-inflammatory cytokines IL-4 and IL-10 (Th2 cells). IFN-γ stimulates the production of immunoglobulin G2a (IgG2a) from B lymphocytes, and IL-4 stimulates production of IgE as well as suppressing IFN-γ-dependent macrophage functions [3]. Whether or not uraemic patients can be
characterized as having predominantly Th1 or Th2 activation defects is still not clear. Logically, they might be expected to suffer mainly from Th1, activation with some Th2 abnormalities.

Monocytes from dialysis patients also produce the pro-inflammatory cytokines IL-6, IL-12 and TNF-α [4–6]. The distinct population of monocytes that produce the anti-inflammatory cytokine IL-10 in healthy people is not, however, present or is observed only infrequently in dialysis patients [5].

Apart from the MIA syndrome, the consequences of chronic inflammation and enhanced immune activation in patients with CKD also include anaemia and resistance to erythropoietic agents, which are the focus of this article; they might also include the development of renal osteodystrophy. In this article, the strategies that might be employed to treat anaemia in patients with CKD who are resistant to erythropoietic agents are discussed.

### Anaemia and erythropoietic resistance

A recent study addressed the role of chronic inflammation and immune activation in haemodialysis patients [7]. It included 18 patients with persistent anaemia [haemoglobin (Hb) ≤10.5 g/dl] despite at least 6 months of high-dose erythropoietin (EPO) treatment, 14 patients who were responding well to EPO treatment (Hb >10.5 g/dl) despite a low dose of EPO, and 14 healthy controls. Mononuclear cells isolated from participants’ whole blood samples were stimulated with phorbol 12-myristate-13-acetate (PMA) and ionomycin in culture. The cells were then examined for cytokine expression by flow cytometry with antibodies against T-cell markers and cytokines, a method found to be highly robust in this patient population.

The T-cell data were particularly interesting given that uraemic patients are known to be in a state of chronic immune activation and given that TNF-α and IFN-γ are the two major cytokines thought to inhibit erythropoiesis (Table 2). CD4+ and CD8+ T-cell expression of TNF-α in patients who had responded well to EPO treatment was the same as that in the healthy controls, but was significantly increased in blood from patients who had shown a poor response to EPO treatment (Figure 1). Similarly, the expression of IFN-γ was significantly increased in both CD4+ and CD8+ T cells from the poor responders compared with both good responders and healthy controls (Figure 2). Expression of the anti-inflammatory cytokine IL-10 was also raised in the poor responders, although it was not increased as much as expression of TNF-α and IFN-γ.

IL-13 is also an anti-inflammatory cytokine, and IL-13 expression by CD4+ T cells was increased in poor responders compared with both good responders and healthy controls. In CD8+ T cells, however, the increase in expression in poor responders was less pronounced and did not reach statistical significance when compared with healthy controls, but was significantly greater than IL-13 expression in the good responders ($P < 0.01$). The expression of IL-4 was the same in all three groups.

How can these differences in cytokine expression be explained and what is their role in EPO resistance? Patients with uraemia with or without other inflammatory conditions could be in a state of chronic immune system activation in which CD4+ and CD8+ T cells are ‘switched on’ to produce pro- and anti-inflammatory cytokines, which in addition to contributing to the chronic state of immune activation might also inhibit erythroid progenitor cell proliferation and antagonize the anti-apoptotic action of EPO (Figure 3). In a similar study, the same group observed that survival of

### Table 1. Causes of enhanced immune system activation in patients with stages 1–5 chronic kidney disease (CKD)

<table>
<thead>
<tr>
<th>CKD stages 1–4</th>
<th>Additional causes in haemodialysis</th>
<th>Additional causes in peritoneal dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced renal clearance of cytokines</td>
<td>Graft and fistula infections</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>Accumulation of advanced glycation end-products</td>
<td>Bioincompatibility</td>
<td>Exit site/tunnel infections</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>Exposure to endotoxins and other cytokine-inducing substances from contaminated dialysate</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unrecognized persistent infections</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Summary of intracellular cytokine staining study in haemodialysis patients who failed to respond to high-dose erythropoietin treatment

<table>
<thead>
<tr>
<th></th>
<th>CD4+ cells</th>
<th>CD8+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Increased ($P &lt; 0.01$)</td>
<td>Increased ($P &lt; 0.05$)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Increased ($P &lt; 0.001$)</td>
<td>Increased ($P &lt; 0.05$)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Increased ($P &lt; 0.01$)</td>
<td>Increased ($P &lt; 0.01$)</td>
</tr>
<tr>
<td>IL-13</td>
<td>Increased ($P &lt; 0.05$)</td>
<td>Increased ($P &lt; 0.01$, but not significant compared with healthy controls)</td>
</tr>
<tr>
<td>IL-4</td>
<td>No increase</td>
<td>No increase</td>
</tr>
</tbody>
</table>

The $P$-values shown are for comparisons between poor and good responders. Cytokine expression did not differ significantly between good responders and healthy controls.

Reproduced, with permission, from Cooper et al. [7].
patients who failed to respond to EPO treatment was
less than that of patients who did respond to treatment (Figure 4) [8].

Strategies for reversing the inflammatory process

Since patients who do not respond well to EPO
treatment exhibit evidence of immune system activation
and inflammation, it is logical to attempt to reverse
the inflammatory process and avoid the apparently
antagonistic effects of the cytokines on the actions of
EPO. By reversing the inflammatory process, mortality
and morbidity might be reduced, as might the incidence
of malnutrition, atherosclerosis, anaemia and possibly
renal osteodystrophy.

Possible strategies for reversing the inflammatory
process can be divided into three types: very specific,
moderately specific and non-specific. Very specific
strategies would target the pro-inflammatory cytokines
directly with monoclonal antibodies. These have been

**Fig. 1.** CD4+ and CD8+ T-cell expression of TNF-α stimulated by
PMA and ionomycin. Reproduced, with permission, from Cooper
et al. [7]. ns, Not significant.

**Fig. 2.** CD4+ and CD8+ T-cell expression of IFN-γ stimulated by
PMA and ionomycin. Reproduced, with permission, from Cooper
et al. [7].
developed to target TNF-α in the treatment of rheumatoid arthritis, but monoclonal antibodies against other cytokines are not yet available for clinical use. Moreover, the tolerability of these agents in patients receiving haemodialysis is as yet untested.

Moderately specific strategies target the T cells and include anti-thymocyte globulin, anti-lymphocyte globulin and the anti-CD3 monoclonal antibody, OKT3. Again, there are serious drawbacks to these approaches that include unacceptable side effect profiles for general use in the EPO-resistant population, the need for parenteral rather than oral therapy, and cost. The only other options to consider at present are the non-specific strategies that target inflammation—steroids, pentoxifylline and thalidomide. Steroids inhibit cytokine production and induce T-cell apoptosis. Thalidomide induces the Th2 response (IL-4) and inhibits Th1 production of IFN-γ. In theory, thalidomide would be the most effective of the three types of treatment, but is unacceptable to many patients because of its history of adverse teratogenic effects. The anti-inflammatory effects of pentoxifylline, a phosphodiesterase inhibitor, were investigated in the studies described below because the drug and its side
Improving erythropoietin treatment outcome in dialysis patients

Effects are well known and, if proven to be beneficial, it would be deemed to be a practical option for dialysis patients.

**Pentoxifylline: a non-specific strategy**

Pentoxifylline has been used to treat peripheral vascular disease for ~20 years. In addition to its potent haemorrhheological and antiplatelet properties, the drug also inhibits monocyte production of TNF-α, and T-cell production of both TNF-α and IFN-γ. Indeed, the anti-TNF-α effect of pentoxifylline has been used to good effect in a number of conditions, including idiopathic dilated cardiomyopathy, childhood type-1 diabetes mellitus, systemic vasculitis and rheumatoid arthritis. Two studies were undertaken to investigate the effect of pentoxifylline as an adjunct to erythropoietic therapy: an open-label study and a double-blind, placebo-controlled study.

**Open-label study**

Persistently poor responders to high-dose recombinant human EPO (>300 U/week) treatment took part in the study [9]. They all had Hb levels of <10.7 g/dl for at least 6 months. Patients were excluded if they had other inflammatory conditions or any other known cause of anaemia. Patients took oral pentoxifylline at a dose of 400 mg once daily. Ex vivo T-cell cytokine production was measured at baseline and after 6–8 weeks, and Hb levels were measured each month for up to 4 months.

Sixteen patients took part in the study and 12 completed it. Of the four patients who dropped out, two were openly non-compliant, one experienced persistent nausea—a recognized side effect of pentoxifylline treatment—and one patient developed confusion that was judged to be unrelated to pentoxifylline treatment [9].

Pentoxifylline reduced ex vivo CD3⁺ T-cell expression of TNF-α after 6–8 weeks in all 12 of the patients who completed the study. Overall, TNF-α expression was reduced by >50% (P=0.007 compared with baseline). IFN-γ expression was similarly reduced (P=0.0002). In contrast, C-reactive protein (CRP) levels, which reflect the generation of pro-inflammatory cytokines [2], were not significantly reduced during 6–8 weeks of treatment and, therefore, in this study, did not prove to be a good marker for this cytokine response.

Median Hb levels rose significantly during 4 months of pentoxifylline treatment (P=0.001). This result is particularly dramatic because some patients had been persistently anaemic for up to a year before taking pentoxifylline, or had Hb levels persistently below 10 g/dl. Indeed, one patient who was dependent upon transfusions no longer needed them because of pentoxifylline treatment, while in another patient the EPO dose had to be reduced [9].

**Double-blind, placebo-controlled study**

This study is ongoing and includes 160 patients with end-stage renal disease receiving either EPO or darbepoetin α treatment. The patients have been stratified into four groups according to Hb levels and EPO dose, as follows: group 1 includes patients with relatively high Hb levels (>11 g/dl) receiving low-dose EPO (<12000 U/week) or darbepoetin α (<60 μg/week) (n=40); group 2 includes patients with relatively high Hb levels (>11 g/dl) receiving high-dose EPO (>12000 U/week) or darbepoetin α (>60 μg/week) (n=40); group 3 includes patients with low Hb levels (<11 g/dl) on low-dose EPO or darbepoetin α (n=40); and group 4 includes patients with low Hb levels (<11 g/dl) receiving high-dose EPO or darbepoetin α (n=40). The study design includes a 3 month run-in period, after which patients are randomized to receive either pentoxifylline (400 mg once daily) or placebo. At the start of treatment and after 2 and 4 months, T-cell expression of cytokines will be measured, as will CRP levels. Hb levels will be measured at monthly intervals from the start of the study and for 7 months after the start of treatment. The randomization code will be ‘broken’ 4 months after the start of treatment, at which point, if appropriate, patients taking pentoxifylline will be allowed to continue taking the drug for another 3 months and patients on placebo will be allowed to switch to pentoxifylline.

**Conclusion**

In conclusion, targeting chronic inflammation or immune activation in patients with CKD may have widespread benefits that include an improved response to erythropoietic agents. Current strategies to target chronic inflammation include either ‘sharp tools’, such as monoclonal antibodies, that cause unacceptable side effects, or ‘blunt tools’, such as pentoxifylline, that are limited in their efficacy. Ideally, of course, sharp tools with a favourable benefit: risk ratio would be used, although none are as yet available that offer a practical solution for patients receiving dialysis. Currently, the only other option available is the use of pyrogen-free, ultra-pure dialysate, which has been shown to prevent increases in IL-6 and CRP expression and improve the response to EPO [10].

Conflict of interest statement. None declared.

**References**


3. Street NE, Mossman TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. *FASEB J* 1991; 5: 171–177


