The role of inflammation in the anaemia of end-stage renal disease

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Abstract
Chronic inflammation is a common feature of end-stage renal disease (ESRD) that is gaining increasing attention as a major cause of morbidity and mortality. It is well established that ESRD per se carries a heightened risk of inflammatory disorders and other co-morbid conditions, but it should also be pointed out that dialysis treatment per se can bring additional risk factors for inflammation, such as impure dialysate or bio-incompatible membranes. Inflammation has recently been associated with atherosclerosis and malnutrition in ESRD, and this link has led to the development of the malnutrition, inflammation, atherosclerosis (MIA) hypothesis. This describes a syndrome whereby raised levels of pro-inflammatory cytokines (such as IL-1, IL-6 and TNF-α) are a common link between malnutrition, inflammation and atherosclerosis. Also, anaemia appears to be an important element linking elevated cytokine levels with poor patient outcomes. Several mechanisms for cytokine-induced anaemia have been proposed, including intestinal bleeding, impaired iron metabolism and suppression of bone marrow erythropoiesis and erythropoietin production. These effects suggest that pro-inflammatory cytokines may also be an important cause of lack of response to recombinant human erythropoietin (rh-Epo) therapy. In the light of this putative role of pro-inflammatory cytokines, anti-cytokine agents may prove useful to optimize efficacy of rh-Epo in anaemic chronic renal failure patients. Other potential therapeutic strategies include minimizing exposure to causes of inflammation from various co-morbid conditions, such as persistent infections and chronic heart failure.

Keywords: anaemia; cytokines; dialysis; epoetin; erythropoietin; inflammation

Introduction
Accumulating recent evidence points to chronic inflammation as a major contributor to morbidity and mortality in end-stage renal disease (ESRD). About 35–65% of ESRD patients receiving haemodialysis (HD) show signs of inflammation, whereas the prevalence in pre-dialysis patients may be somewhat lower [1]. Several factors have been implicated as potential causes of inflammation in patients with ESRD (Table 1). These include impaired renal clearance of cytokines, accumulation of advanced glycation endproducts (AGEs), atherosclerosis per se and other inflammatory diseases, and unrecognized persistent infections, such as Chlamydia pneumoniae and dental/gingival infections. In addition, the dialysis procedure per se has been linked to an increased risk of inflammation, and available evidence suggests that the prevalence of elevated serum levels of C-reactive protein (CRP) increases somewhat when patients start dialysis [2].

The link between dialysis and inflammation may be due to three main mechanisms. First, dialysed patients are susceptible to infections: local graft and fistula infections due to HD, or peritonitis caused by peritoneal dialysis (PD). Secondly, there is evidence that bio-incompatible dialysis membranes may induce an inflammatory response: they have been linked both with raised IL-6 levels and with increased IL-6 release by monocytes [3,4]. Thirdly, dialysed patients may be exposed to contaminated dialysate containing cytokine-inducing substances such as endotoxins.

Thus, there is a well-documented link between inflammation and ESRD. In addition, elevated levels of pro-inflammatory cytokines are associated with poor patient outcomes [5,6], which may in part be due to anaemia. This paper will review some of the likely mechanisms for this link, and discuss implications for the management of inflammation-related anaemia in ESRD patients.

The MIA hypothesis
An interesting paradox in ESRD is that although markers of inflammation and malnutrition are strong
predictors of mortality, infectious diseases do not very often lead to death in this patient group. Similarly, markers of malnutrition such as low serum albumin also predict mortality, but less than 5% of ESRD patients actually die from this cause [1]. It is well recognized that atherosclerotic cardiovascular disease is the most frequent cause of death in this patient population. So how may this paradox be best explained? One possible explanation may be the strong documented association between malnutrition, inflammation and atherosclerosis (MIA) in ESRD [2]. Several researchers have linked both malnutrition and inflammation with an increased risk of atherosclerotic cardiovascular disease (itself an inflammatory disorder). These findings have led us to suggest the existence of a syndrome consisting of MIA in some patients with ESRD [7]. Indeed, inflammation is almost invariably accompanied by atherosclerosis and/or malnutrition in ESRD patients, according to a recent study of 109 predialysis patients (Figure 1) [1,2]. This study revealed that 32% showed signs of inflammation, but only 1% showed signs of inflammation without concomitant atherosclerosis or malnutrition.

**Pro-inflammatory cytokines in ESRD**

It seems likely that pro-inflammatory cytokines are a central link in the vicious circle of malnutrition, inflammation and atherosclerotic cardiovascular disease. Patients with renal disease appear to have an up-regulated pro-inflammatory cytokine system associated with a range of infectious and inflammatory stimuli as outlined above. Actually, recent data suggest that levels of pro-inflammatory cytokines in HD patients are 8–10-fold higher than in healthy controls [6].

Several studies have linked high levels of pro-inflammatory cytokines with poor outcomes in renal patients. In one study of 90 HD patients followed for 1000 days, IL-6 was the strongest predictor of death (Figure 2) [5]. This supports the findings of Kimmel et al. [6] in 230 HD patients, linking IL-6 and other cytokines released by activated monocytes with poor outcome. In contrast, high levels of cytokines associated with better T-cell function, such as IL-2 and IL-12, were associated with a survival advantage for these patients [6]. Further evidence comes from a long-term follow-up of 111 dialysis patients showing that 5-year survival is dramatically reduced in the 25% of patients whose serum IL-6 levels are the highest (Stenvinkel et al. unpublished data).

Elevated levels of pro-inflammatory cytokines are associated with behavioural, nutritional and physiological adaptations, many of which are characteristic of symptoms displayed by ESRD patients [8]. Behavioural adaptations include fatigue, altered sleep pattern, malaise and anorexia. At the same time, nutritional adaptations such as weight loss, negative nitrogen balance, hyperinsulinaemia, hypertriglyceridaemia and hypercholesterolaemia occur. Patients also experience physiological adaptations such as raised resting energy expenditure, stress hormone response, skeletal muscle wasting, increased acute phase response, impaired gastric emptying, bone marrow suppression, iron metabolite modulation and intestinal bleeding [8].

### Pro-inflammatory cytokines and anaemia

Anaemia may be one of the many links between raised pro-inflammatory cytokines and poor outcomes in renal patients. Indeed, several potential mechanisms linking cytokines with anaemia have been proposed, and a complex picture of interactions between different cytokine effects is emerging. These mechanisms include bone marrow suppression of erythropoiesis, reduced erythropoietin secretion, intestinal bleeding and disrupted iron metabolism.

#### Impaired bone marrow function

Probably the most important mechanism for pro-inflammatory cytokine-induced anaemia is suppression of bone marrow erythropoiesis. IL-1, TNF-α and IFN-γ have all demonstrated suppressive effects on erythropoiesis, and this mechanism probably plays a central role in the pathogenesis of anaemia in patients with inflammation [9,10]. Moreover, IL-1 and IL-6 can antagonize erythropoietin’s ability to stimulate bone marrow proliferation in culture [11]. In contrast, another study detected no evidence for bone marrow suppression during twice daily intraperitoneal injection of IL-6 in rats [12]. Likewise, Pojda et al. [13] have

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**Table 1. Potential causes of inflammation in patients with ESRD**

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<thead>
<tr>
<th>ESRD</th>
<th>Additional causes in HD</th>
<th>Additional causes in PD</th>
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<tbody>
<tr>
<td>Reduced renal clearance of cytokines</td>
<td>Graft and fistula infections</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>Accumulation of AGEs</td>
<td>Bio-incompatibility</td>
<td>Bio-incompatibility</td>
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<tr>
<td>Chronic heart failure</td>
<td>Exposure to endotoxins and other cytokine-inducing substances from contaminated dialysate</td>
<td>Exposure to endotoxins and other cytokine-inducing substances from contaminated dialysate</td>
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<tr>
<td>Atherosclerosis per se</td>
<td>Various inflammatory diseases</td>
<td>Unrecognized persistent factors</td>
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shown that daily IL-6 injections actually increased the number of progenitor cells in the bone marrow of normal mice.

Interestingly, other cytokines such as IL-12 and insulin-like growth factor 1 (IGF-1), enhance bone marrow progenitor cell proliferation [14,15]. Also, IL-3 may boost erythropoiesis by promoting stem cell differentiation to form primitive erythroid cells [16,17]. Thus, it seems likely that the impact on erythropoiesis depends upon a balance between different cytokines, rather than on absolute levels of various pro-inflammatory cytokines taken in isolation.

Cytokines may inhibit erythropoietin production

Patients with anaemia of chronic disorders have inappropriately low levels of erythropoietin for their degree of anaemia [18], and it has been postulated that pro-inflammatory cytokines could cause anaemia by inhibiting erythropoietin secretion. This idea gains support from in vitro evidence that IL-1α, IL-1β and TNF-α inhibit erythropoietin production in a culture of the human hepatoma line G2 [19]. However, in another study, no difference was found between rh-Epo responses in anaemic rats with or without acute inflammation [20].

Intestinal bleeding

Another potential mechanism for anaemia due to pro-inflammatory cytokines is increased bleeding through the intestinal wall. A Dutch study of IL-6-induced anaemia demonstrated significant intestinal blood loss in rats given intraperitoneal IL-6 injections for 14 days, compared with controls [12]. Further evidence suggests that TNF-α may mediate intestinal injury; for example it plays a critical role in the pathogenesis of gastric injury induced by non-steroidal anti-inflammatory drugs (NSAIDs). In this respect, it is of interest that a TNF-α inhibitor, pentoxiphilin, dose-dependently reduced NSAID-induced gastric damage in rats, supporting the idea that increased pro-inflammatory cytokine levels are one cause of the high prevalence of intestinal bleeding seen in renal patients [21]. In vivo studies have also demonstrated
that intravascular injections of rh-TNF-α can lead to watery diarrhoea, vascular leak syndrome and necrosis of the villi [22], as well as an acute inflammatory response with haemorrhage in the caecum [23].

**Iron metabolism**

There appears to be a positive relationship between ferritin levels and markers of inflammation such as CRP, suggesting that ferritin may act as an acute phase reactant. Indeed, cytokines might induce ferritin synthesis at the transcriptional level, and also promote iron uptake by hepatocytes (Stenvinkel et al. unpublished data). Thus, increased ferritin production and impaired transferrin production due to inflammation prevents iron delivery to erythrocyte precursors by shunting iron to the reticulo-endothelial storage pool. However, as a recent Dutch study has demonstrated that mucosal uptake and transfer of iron was much lower in patients with inflammatory disease than in patients without an acute phase response [24], inflammation may also impair iron availability by reducing absorption of dietary iron.

**Implications for anti-anaemia therapy**

The presence of inflammation, with an associated rise in serum levels of pro-inflammatory cytokines, may be the second most common cause of resistance to erythropoietin [25]. Indeed, dialysis-related cytokine induction has been shown to blunt the response to erythropoietin in two recent clinical studies. In the first study, a Spanish group measured monocytic cytokine levels in 34 HD patients [26]. Their results linked raised IL-6 and TNF-α levels with an increased rh-Epo requirement in renal patients receiving HD (Figure 3). The same study also demonstrated a negative correlation between IL-12 levels and effective rh-Epo dose, suggesting that it is the balance between different cytokines—rather than absolute cytokine levels—that determines their effect on erythropoiesis. More recently, a 12-month prospective controlled study of 30 male HD patients in Germany showed an association between increased rh-Epo requirements and dialysis-induced rises in IL-6 levels [27]. This study also highlighted the importance of using ultra pure dialysate to minimize inflammation, and so optimize response to rh-Epo, in ESRD patients. The authors assigned patients to conventional or ultra pure dialysate, and found that both CRP and IL-6 levels were significantly reduced in the group receiving ultra pure dialysate ($P<0.05$).

**Therapeutic approaches**

Many authors recommend increasing the rh-Epo dose by 30–70% to achieve target haemoglobin levels in iron-replete patients with inflammation. It should be noted, however, that high doses of rh-Epo could lead to functional iron deficiency in patients with chronic inflammation, by stimulating erythropoiesis so much that it exceeds the maximal capacity to deliver iron. One way to address this problem might be to supplement these patients' dietary iron supply. However, it is recommended that intravenous iron should be discontinued in patients with bacteraemia because iron may promote bacterial growth. In contrast, low dose iron has been shown to be safe and effective in patients with systemic inflammation of non-bacterial aetiology who have functional iron deficiency [28].

It is also important to monitor patients for chronic infections and inflammatory disorders, and treat these conditions accordingly. It should also be noted that optimal dialysis treatment, using ultrapure dialysate and biocompatible membranes, should be used in order to avoid further inflammatory triggers for these patients.

Research to date has opened up new possibilities for future therapy of inflammation-related complications, such as anaemia, atherosclerosis and malnutrition. Potential treatment strategies include selective anti-cytokine therapy to restore the balance between pro-inflammatory cytokines and other cytokines that promote erythropoiesis. Agents such as anti-TNF-α antibodies, soluble TNF receptors, and IL-1 and IL-6 receptor antagonists have already achieved dramatic results in other patient groups with wasting syndromes, such as chronic heart failure and rheumatoid arthritis. The concept of immunonutrition is also rapidly gaining support, as vitamin E supplementation has been shown to lower CRP and monocyte IL-6 levels in healthy controls and patients with type 2 diabetes [29]. Finally, it may in the future be possible to develop new treatment strategies that combat inflammation and the related anaemia by manipulating the NF-κB system [30].

**Conclusion**

Chronic inflammation is a common feature of ESRD and a range of different causes—both related and
unrelated to dialysis—have been identified. Inflammation is linked with an increased risk of morbidity and mortality for both pre-dialysis and dialysis patients. Raised levels of pro-inflammatory cytokines (TNF, IL-1 and IL-6) have been linked to poor outcomes via a number of mechanisms, including anaemia. Furthermore, inflammation may be one of the most important reasons for lack of response to rh-Epo in anaemic ESRD patients. Thus, minimizing inflammation and its consequences can be an effective strategy to reduce anaemia in these patients.

References