The inflammatory response and epoetin sensitivity

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Abstract
Patients receiving epoetin therapy show wide variability in their responsiveness to the drug. Many factors may be responsible for this, particularly iron deficiency, acute infection and under-dialysis. Even after excluding factors known to cause resistance to epoetin, the marked variability in sensitivity to epoetin remains. The exact mechanism of this effect is unclear. It is, however, recognized that uraemia is a chronic inflammatory state, with some patients showing quite significantly increased laboratory markers of inflammation and immune activation. It is also known that chronic inflammation can modify the process of erythropoiesis, and this is probably mediated via pro-inflammatory cytokines such as interleukin-1 (IL-1), tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interferon-\(\gamma\) (IFN-\(\gamma\)). It is hypothesized, therefore, that some patients showing resistance to epoetin may have enhanced levels of immune activation, causing increased release of pro-inflammatory cytokines in the bone marrow. This has been investigated by studying T-cell phenotypes by flow cytometry, along with cytokine release from T cells and monocytes in ‘good’ and ‘poor’ responders to epoetin. Poor responders were found to have significantly reduced CD28 expression on both CD4\(^+\) and CD8\(^+\) cells, enhanced IL-10 generation from peripheral blood mononuclear cells (PBMCs), higher plasma IL-12 levels and enhanced TNF-\(\alpha\) release from PBMCs. The patients in this study, who were followed-up for the subsequent 24 months, had a considerably lower survival if they were poor responders (54\% vs 88\% for good responders; \(P<0.05\)). Further work in this area is required to confirm or contest the hypothesis that epoetin resistance is due to enhanced levels of immune activation.

Keywords: anaemia; chronic inflammation; drug sensitivity; epoetin; erythropoietin; uraemia

Introduction
Much experience has been gained in the use of recombinant human erythropoietin (r-HuEPO, epoetin) in the treatment of renal anaemia over the last 15 years. The majority of patients receiving this drug respond satisfactorily with a significant rise in haemoglobin concentration, and this is associated with documented improvements in quality of life, exercise capacity and cardiac function. Approximately 5–10\% of renal patients receiving epoetin, however, show a suboptimal response to the drug, either by failing to attain their target haemoglobin despite high doses of epoetin or by the loss of a previous satisfactory response to the drug [1]. Even in patients who do respond to epoetin, there is an enormous variability in sensitivity to the drug, perhaps as much as 10-fold. Many factors have been recognized as causing some resistance to epoetin, notably iron deficiency, acute infection and under-dialysis. There are also other less prominent causes of a poor response to epoetin, such as hyperparathyroidism, aluminium toxicity, B\(_12\) or folate deficiency, haemolysis, bone marrow disorders, haemoglobinopathies, carnitine deficiency, concomitant angiotensin-converting enzyme (ACE) inhibitor therapy and (very rarely) antibodies against the erythropoietin molecule [1–4].

Even after excluding all these factors (as much as is feasible), the marked variability in sensitivity to epoetin remains, and the exact mechanism for this effect is unclear. The aim of this article is to present a hypothesis that may increase our understanding of the mechanisms involved.

Uraemia and inflammation
It has been recognized for the last few years that uraemia is a chronic inflammatory state [5]. Thus, even in the absence of overt infection, many patients with renal failure show increased levels of acute phase proteins such as C-reactive protein (CRP), ferritin, fibrinogen and interleukin-6 (IL-6) [6,7]. This is mirrored by low serum albumin levels, enhanced lipid
peroxidation and increased oxidative stress [8,9]. Such patients show clinical features of malnutrition, inflammation and enhanced atherosclerosis, leading to the postulation of the ‘malnutrition, inflammation and atherosclerosis’ (MIA) syndrome [10]. It has been shown consistently that patients with high CRP levels or low albumin levels have a much reduced survival compared with those with normal levels of inflammatory markers [11,12].

Uraemia and erythropoiesis

Since the 1970s, it has been recognized that uraemia suppresses erythropoiesis [13–16], and this has been postulated as contributing to the pathogenesis of renal anaemia. For example, incubation of uraemic serum with erythroid progenitor cells leads to inhibited proliferation. It has been assumed that there are factors circulating in uraemic plasma which have a direct effect on the proliferation of erythroid progenitor cells, and putative substances have included spermine, spermidine, putrescine and parathyroid hormone [15,16]. Which factors, if any, play a major part in the disease process remains unclear, but it is known that increasing the intensity of dialysis in uraemic patients can improve erythropoiesis [17].

Inflammation and erythropoiesis

Even in the absence of uraemia, chronic inflammatory states are associated with anaemia, and this has been termed the anaemia of chronic disease. For example, conditions such as rheumatoid arthritis, polymyalgia rheumatica and malignancy are associated with low haemoglobin levels. Much work has been done in this area to elucidate the pathogenesis of this condition, and it has become apparent over the last few years that this is probably mediated via pro-inflammatory cytokines such as IL-1, tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [18,19]. Such cytokines are known to be released more readily in chronic inflammatory states, and in vitro studies indicate potent suppression of erythroid colony growth. IFN-γ, in particular, has been shown to promote apoptosis of erythroid progenitor cells, thus antagonizing the anti-apoptotic action of erythropoietin [19].

The hypothesis

Based on the observations described above, it is hypothesized that variation in epoetin sensitivity can be explained by the presence of chronic inflammation. This is supported by data from the European Survey on Anaemia Management (ESAM), which showed lower haemoglobin levels, despite high doses of epoetin, in the 201 patients who had a CRP level ≥50 mg/dl on at least three of the six monthly visits, compared with 3014 patients with a CRP level <50 mg/dl on at least four of the six visits (Figure 1) [20]. In a study by Allen et al. [21], bone marrow cell cultures incubated with autologous serum from patients with uraemia plus inflammation produced greater suppression of CFU-E colony growth compared with the effect of serum on the bone marrow cultures obtained from uraemic controls with no inflammation (Figure 2). The mechanism for this effect was investigated in a similar co-culture experiment with the addition of neutralizing polyclonal antibodies against TNF-α and/or IFN-γ. The presence of both antibodies independently reversed the suppression of erythropoiesis in the uraemic inflammatory patients and, interestingly, co-incubation with both antibodies completely reversed the suppression of CFU-E colony growth in the uraemic control patients. No effect on erythropoiesis was seen with the addition of control antibody (goat IgG). These experiments suggest a role for pro-inflammatory cytokines in suppressing erythropoiesis, and possibly altering the sensitivity to epoetin. It was hypothesized that

![Graph of Epoetin dose and haemoglobin levels in patients with chronic infection/inflammation](http://ndt.oxfordjournals.org/)

**Fig. 1.** Epoetin dose and haemoglobin levels in patients with chronic infection/inflammation. (Reproduced with permission from [20].)
patients requiring higher doses of epoetin have more 'inflammatory activity'.

Investigation of 'good' and 'poor' responders to epoetin

To investigate this hypothesis further, two groups of haemodialysis patients in our unit were studied: 18 patients who were responding well to epoetin (haemoglobin >10 g/dl and a mean (±SD) epoetin dose of 71±30 U/kg/week) and 15 poor responders (haemoglobin <10 g/dl, mean (±SD) epoetin dose 396±147 U/kg/week). Patients had all the common causes of resistance to epoetin excluded, such as iron deficiency, overt infections or blood loss, underdialysis, hyperparathyroidism, etc. All patients had been maintained on the same dose of epoetin for at least 3 months prior to investigation. Diabetic patients were excluded. A third group of normal healthy subjects was also studied to yield control data.

The actual haemoglobin levels and epoetin doses in the patients are shown in Figure 3. There was a clear separation between the good and poor responders to epoetin. Plasma cytokine levels were measured in the three subject groups, and particular interest was directed at the key pro-inflammatory cytokines, such as IFN-α, IFN-γ and TNF-α. There were, however, no measurable levels of these cytokines in the patients’ plasma (Table 1). Plasma IL-6 levels were increased in uraemic patients, and there was a stepwise increase in the CRP level from normal controls (4.0±3.4 mg/l) to good responders (8.5±7.8 mg/l) to poor responders (21.2±22.7 mg/l). This again suggests that inflammation is playing a part in affecting the response to epoetin, even in the absence of any obvious infection or inflammatory condition.

It is likely that the biological action of the cytokines is occurring at a local level in the bone marrow microenvironment, which may explain why the plasma cytokine levels were unremarkable. Pro-inflammatory cytokines originate from T cells and monocytes. In view of the difficulty in obtaining bone marrow samples from the patients in this study (particularly the good responders), peripheral blood mononuclear cells (PBMCs) were obtained following density gradient centrifugation of whole blood by Ficoll-Hypaque. The T cells were then examined for their phenotype by flow cytometry, and cytokine secretion from T cells was assessed in an unstimulated state and after stimulation with monoclonal antibodies to CD3 and CD28.

Preliminary results revealed a reduction in CD28 expression in CD4 and CD8 cells in poor responder patients as compared with good responders and controls (Figure 4). Spontaneous IL-10 generation from PBMCs was significantly greater in poor responders compared with good responders (P<0.05), and there was a tendency towards higher IL-10 levels after stimulation with CD3/CD28 monoclonal antibodies in the poor responder group. Serum neopterin levels were raised in both good and poor responders compared with normal controls, but there was no difference between the two groups of uraemic patients. Plasma
IL-12 levels were significantly higher in poor responders compared with good responders ($P<0.02$), and unstimulated TNF-α release tended to be higher in poor responders compared with good responders.

Although analysis of mortality was not initially part of the study, it became apparent that survival was reduced for patients who responded poorly to epoetin compared with those who responded well. The survival curves for the two groups of patients were therefore assessed over the 2 years since the patients originally were studied, and there was a significant difference between the 18 good responders (survival 88% at 24 months) vs the 15 poor responder patients (survival 54% at 24 months; $P<0.05$) (Figure 5).

Conclusions

This preliminary work suggests that patients with uraemia and other inflammatory conditions have enhanced immune activation, involving T cells and monocytes (Figure 6) [22,23]. These T cells and monocytes secrete a number of pro-inflammatory cytokines, with one of the major effects being enhanced local production of IFN-γ. Monocytes, when activated, produce higher levels of IL-12, which is known to stimulate Th1 cells, which in turn produce IFN-γ [24]. In addition, monocytes produce increased levels of TNF-α, which may promote the action of IFN-γ as a pro-apoptotic agent [25]. The effect of IFN-γ in the bone marrow is to cause the early death of erythroid progenitor cells, thereby antagonizing the anti-apoptotic action of epoetin [19]. In addition, the production of inflammatory cytokines from monocytes may also interfere with iron metabolism, thereby reducing iron availability to the bone marrow and causing a condition known as functional iron deficiency. This, in turn, will exacerbate the resistance to epoetin.

It should be emphasized that this hypothesis is still preliminary, and further supportive work is necessary before this can be offered as a definitive explanation. If proven to be valid, the hypothesis would explain why patients with inflammatory disease fail to show a good response to epoetin, and why patients vary in their sensitivity to epoetin even when no obvious inflammation is present. If confirmed, then it may be possible to modify this disease process by the use of specific anti-cytokine antibody therapy. In the meantime, it may be possible to reduce cytokine induction by altering the dialysis regimen. Sitter et al. [25] showed that the use of ultrapure dialysate for 1 year was associated with a significant reduction in CRP and IL-6 levels, producing a better response to epoetin therapy in 30 male haemodialysis patients, when compared with the conventional dialysate used before starting the study.

To summarize, a hypothesis has been generated which suggests that some patients showing resistance to epoetin might have enhanced activity of T cells and monocytes, with the concomitant production of pro-inflammatory cytokines in the bone marrow. These cytokines, in turn, might act locally to antagonize the action of epoetin at a cellular level, thereby causing
resistance to epoetin treatment. It may be possible to modify this process by altering the dialysis prescription, and the use of specific anti-cytokine therapies may be a possible strategy in the future.

References


Fig. 6. The hypothesis: how enhanced immune activity can result in epoetin resistance.