Inflammation and resistance to erythropoiesis-stimulating agents—what do we know and what needs to be clarified?

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Abstract
Resistance to erythropoiesis-stimulating agents (ESA) in patients with chronic kidney disease (CKD) can be associated with evidence of enhanced systemic inflammatory responses. This review considers the inflammatory mechanisms thought to be involved in the development and aetiology of anaemia of CKD that may help our understanding and management of patients with ESA resistance. The potential role of nutritional support and of anti-inflammatory therapies in managing resistance to ESA therapy is discussed and explored.

Keywords: anaemia; darbepoetin; epoetin; hyporesponse; inflammation; resistance

Introduction
Anaemia associated with chronic kidney disease (CKD) develops gradually during progressive decline of renal function and is characterized by a relative deficiency of erythropoietin secretion from the diseased kidney in relation to the degree of anaemia. More than 90% of patients with anaemia of CKD respond adequately to erythropoiesis-stimulating agents (ESAs) at doses of <20 000 IU/week (epoetin) or <100 µg/week (darbepoetin-α) [1]. However, a growing list of factors appear to influence the response to ESAs, including the mode of drug administration, type of renal replacement therapy, functional or absolute iron deficiency, systemic inflammatory factors and secondary hyperparathyroidism [2–5]. This review focuses on inflammatory-mediated aspects of the responsiveness to ESA treatment in CKD patients.

Defining inadequate response to ESAs—the contribution of inflammation to hyporesponse
The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) Guidelines define an inadequate response to ESAs as a failure to achieve target haemoglobin (Hb)/haematocrit (Hct), in the presence of adequate iron stores, with doses of epoetin of 450 IU/kg/week intravenously (i.v.) or 300 IU/kg/week subcutaneously (s.c.), within 4–6 months of treatment initiation, or a failure to maintain target Hb/Hct subsequently at these doses [6]. Similarly, the revised European Best Practice Guidelines define ESA resistance as a failure to attain the target Hb levels while receiving >300 IU/kg/week (~20 000 IU/week) of epoetin or 1.5 µg/kg of darbepoetin-α (~100 µg/week) or as the continued need for these ESA doses in order to maintain the target [1].

In observational studies of dialysis patients, the need for high ESA doses is strongly associated with a more severe anaemia [7,8]. Patients requiring high doses of ESA also have significantly higher levels of inflammatory markers such as C-reactive protein (CRP) or interleukin-6 (IL-6) [7,9,10], with these inflammatory markers predicting anaemia and accounting statistically for ~10% of the overall variation in ESA dose requirements [10,11].

How should inflammation be diagnosed and monitored in CKD patients?
The typical acute phase response to systemic inflammation induces characteristic rises in the circulating levels of several proteins, including CRP and serum...
amyloid A protein, which can increase as much as 100- to 1000-fold in response to severe infections. Other positive acute phase proteins that rise during inflammation are complement factors, fibrinogen and haptoglobin. Negative acute phase proteins such as albumin decrease during inflammation [12–14]. In clinical practice, measurement of CRP levels is widely used to monitor inflammation. Assay of CRP offers a reliable and readily available test that is simple, sensitive and inexpensive. However, it is a non-specific indicator of inflammation that does not differentiate either between infection and other underlying causes of inflammation or between acute and chronic inflammation. Although direct measurement of other cytokines can be performed using commercial assays, to date high costs and a lack of standardization have restricted the use of these tests to clinical studies.

Mechanisms of inflammatory-induced ESA resistance

Elevated cytokine levels and enhanced oxidative stress are implicated in the development of anaemia. Accumulating evidence suggests that activation of acute phase responses and the cytokine cascade occurs in patients with CKD [9,11,15]. Activation of the immune system diverts iron traffic from erythropoiesis to storage sites within the reticuloendothelial system, inhibits erythroid progenitor proliferation and differentiation, suppresses erythropoietin production, blunts response to erythropoietin and accelerates destruction of erythrocytes coated with immune complexes or immunoglobulins [16].

Pro-inflammatory cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) diminish colony formation of burst-forming unit-erythroid cells (BFU-Es) and colony-forming unit-erythroid cells (CFU-Es) [17,18]. IFN-γ appears to be the most potent inhibitor of CFU-E proliferation, probably accounting for the inverse correlation between plasma IFN-γ levels and reticulocyte count [19]. It has been demonstrated that serum derived from uraemic patients suppresses the normal erythroid colony-forming responses to erythropoietin in a manner that can be inhibited by antibodies against IFN-γ and TNF-α, suggesting a key role for these inflammatory mediators in uraemia [20]. It has also been demonstrated that high circulating levels of blood soluble receptor p80 for TNF-α are associated with ESA resistance in haemodialysis patients [21]. These marked inhibitory effects of IFN-γ and TNF-α on erythroid progenitor cells might be related to the ability of these cytokines to generate nitric oxide (NO) [22], a substance that is known to suppress the proliferation of erythroid progenitor cells.

In poor responders to ESA, there is increased expression of CRP and of erythropoiesis-inhibiting cytokines such as TNF-α, IFN-γ, IL-10 and IL-13 by T cells [20,23]. Data from in vitro studies also support a relationship between high levels of IFN-γ or TNF-α and a need for increased doses of erythropoietin to effect and restore CFU-E colony formation [24].

Diversion of iron traffic from erythropoiesis to storage in chronic inflammation

Activation of the cytokine cascade and associated acute phase responses induce alterations in iron metabolism. Hypoferaemia occurs because of enhanced iron retention and storage within the reticuloendothelial system during inflammation, and this limits the availability of iron for erythropoiesis. Studies in mice have shown that pro-inflammatory cytokines derived from Th1 (T-helper 1) lymphocytes provoke both hypoferaemia and anaemia, and that recombinant TNF-α and recombinant IL-1 cause a significant decrease in serum iron levels [25]. In humans, administration of recombinant human TNF-α or recombinant human IFN-γ results in hypoferaemia accompanied by an increase in circulating ferritin concentration and a decrease in soluble transferrin receptor levels [26,27]. There are also in vivo data to suggest that pro-inflammatory cytokines or lipopolysaccharides (LPS) can alter the regulation of several genes involved in iron uptake, storage and utilization in various cell types [28].

Increased expression of ferritin and enhanced iron sequestration

During development of erythroid and other cells, two iron-regulatory proteins (IRP-1 and IRP-2) act to control the level of the intracellular labile iron. These iron-regulating proteins act at the translational level to regulate synthesis of the transferrin receptor (divalent metal transporter 1, which determines iron uptake by the cells) and of ferritin (which determines intracellular iron sequestration) [29,30].

NO and reactive oxygen species

Recent research supports the concept that cytokine-induced alterations in cellular iron homeostasis are mediated in part by the increased production of NO [28,31–39] and reactive oxygen species [40,41]. In macrophages exposed to LPS and IFN-γ, a dramatic increase in ferritin synthesis can be observed [28,37,39], and several laboratories have reported that NO enhances the IRE (iron response element)-binding activity of IRP-1 in various cell types [31–36]. Moreover, the NO-mediated increase in IRP-1 activity has been shown by some investigators to be accompanied by translational repression of ferritin synthesis in macrophages and other cells [31–33,35,36]. However, these data are in conflict with many other studies demonstrating that inflammatory cytokines, which are known to induce NO production in macrophages, actually stimulate ferritin synthesis [28,37–39]. These paradoxical results might be explained by the fact that
NO exhibits markedly different biological effects depending upon its redox state. NO in its reduced form, NO\textsuperscript{−}, has high affinity for iron and alters the [Fe–S] cluster of IRP-1, resulting in high binding activity for IRE [31,33,34]. In contrast, the oxidized form of NO (NO\textsuperscript{+}, nitrosonium ion) causes S-nitrosoylation of thiol groups in IRP-2 and IRP-1, thereby markedly decreasing the IRE-binding activity of IRPs [28,37–39], which in turn leads to increased ferritin synthesis. In macrophages of the reticuloendothelial system, cytokine-induced degradation of IRP-2 mediated by NO might be responsible for the dramatic upregulation of ferritin synthesis and the resulting increased sequestration of intracellular iron [28].

Pro- and anti-inflammatory processes

It is intriguing to note that not only pro-inflammatory but also anti-inflammatory cytokines play major roles in the regulation of intracellular iron homeostasis [36]. In activated murine macrophages, IL-4 and IL-13 increase ferritin translation by opposing the binding activity of IRPs for IRE in the 5' untranslated region of ferritin mRNAs. These anti-inflammatory cytokines are thought to suppress NO formation, and may also alter its redox state, favouring the formation of the oxidized form of NO. Thus, T-helper 2 (Th2) lymphocyte-derived anti-inflammatory cytokines are able to increase iron sequestration in activated macrophages and may also contribute to the development of anaemia [36].

Reactive oxygen species such as O\textsubscript{2}− (superoxide anion) and H\textsubscript{2}O\textsubscript{2} (hydrogen peroxide) are potentially toxic by-products of aerobic metabolism. Studies in liver lysates have shown that superoxide anion or hydrogen peroxide alone lack reactivity toward IRP-1, but the combined action of these two reactive species can induce reversible inactivation of IRP-1 for IRE [41].

In contrast, an exogenous pulse or an enzymatic source of hydrogen peroxide has been shown to provoke a rapid increase in IRP-1 activity in cells or tissues [40]. Data suggest that IRP-2 is also highly sensitive to oxidative modification by endogenous reactive oxygen and is accompanied by proteasome-mediated degradation of this regulatory protein [42]. However, the binding activity of IRP-2 is not affected by exposure to exogenous hydrogen peroxide. Thus, on balance, there is evidence that oxidative stress during inflammation may alter the expression of ferritin synthesis at a translational level and that, under certain conditions, transcriptional mechanisms are also affected [43].

Cytokine-mediated alteration in the IRE-binding activity of IRPs, and the diminished availability of apotransferrin during inflammation, leads to reduced iron transport into the portal circulation. Indeed, impaired mucosal absorption of iron is seen in haemodialysis patients with increased CRP levels [44].

According to recent data, a key factor in the development of functional iron deficiency may be the IL-6-induced production of hepcidin in the liver. Hepcidin is a peptide that is a negative regulator of iron absorption in the small intestine and iron release from macrophages [45]. High levels of hepcidin have been found in patients with anaemia of chronic diseases and in CKD patients with anaemia [46,47].

What are the roles of nutritional support, vitamins and co-factors in anaemia treatment?

End-stage renal disease patients often have a low protein and energy intake, which may affect the level of nutrients essential to erythropoiesis and also contribute to a shorter erythrocyte life span [48]. In non-renal populations, it is known that severe malnutrition is associated with anaemia, which can be reversed by nutritional intervention [49]. CKD patients with a low body mass index (BMI) are more likely to suffer from severe anaemia than obese patients, who appear to have higher leptin levels [50–52]. Indeed, obesity has been associated with reduced ESA needs (per kg body weight) in the management of anaemia. However, to date, there are no data for anaemic CKD patients to suggest a direct clinical benefit of management with protein- and energy-rich nutritional supplements, although there are early reports that amino acid ketoanalogues may help improve anaemia in dialysis patients [53].

Anti-inflammatory and/or anti-oxidative agents with potential effects on ESA resistance

The body produces a wide array of natural anti-oxidants in an attempt to curtail the harmful effect of reactive oxygen species. Endogenous enzymatic anti-oxidants include superoxide dismutase, catalase and glutathione peroxidase [54,55]. Each of these enzymes reduces the reactivity of reactive oxygen species; superoxide dismutase catalyses the dismutation of O\textsubscript{2}− to H\textsubscript{2}O\textsubscript{2}, catalase reduces H\textsubscript{2}O\textsubscript{2} to water, and selenium-containing glutathione peroxidase reduces organic lipid peroxides in the presence of glutathione [55]. Other, non-enzymatic anti-oxidative agents, such as glutathione, vitamin E, vitamin C, transferrin and albumin [54], act as scavengers for reactive species such as H\textsubscript{2}O\textsubscript{2}, OH− (hydroxyl anion) and chlorinated oxidants, and prevent lipid peroxidation.

A number of different observations and studies attest to the anti-oxidant properties of vitamins. Vitamin E has been shown to reduce CRP and monocyte IL-6 levels in healthy volunteers and in patients with diabetes mellitus type 2 [56]; it is also known to inhibit the activation of protein kinase C and nuclear factor-κB by reactive oxygen species [57]. A recent study demonstrated that γ-tocopherol and its metabolite may exert a significant anti-oxidative activity [58], and coating of dialyser membranes with vitamin E has been shown to reduce oxidative stress...
Vitamin C also appears to be beneficial for liberation of iron from its stores [63], and improves the response to ESA in iron-overloaded patients [64,65]. However, although a daily vitamin C dose of 150 mg is usually considered safe [66], i.v. vitamin C treatment must be administered with caution during anaemia since high doses may result in oxalate accumulation (especially in case of vitamin B6 deficiency).

Glutathione has anti-oxidant properties and has been reported to prolong erythrocyte survival, improve anaemia and reduce the need for ESA for dialysis patients when used either alone [67,68] or in combination with vitamin E-coated dialysers [69].

Melatonin is thought to prevent defective mitochondrial oxidative phosphorylation by stimulation of complex I and IV of the electron transport chain enzymes in mitochondria [70]. This endogenous substance has been reported to reduce the oxidative stress induced by i.v. iron and ESA therapy [71]. Omega-3-fatty acids have not been shown to have a positive effect on anaemia [72]. Other compounds that have been studied for their anti-oxidant properties, such as selenium and the statin class of lipid-lowering drugs, have not been tested in the context of renal anaemia.

Cytokines or their antagonists have been used successfully in the management of many inflammatory diseases and wasting syndromes and, currently, monoclonal antibodies such as anti-TNF-α antibodies, soluble TNF-α receptors, IL-1 and IL-6 receptor antagonists are available as therapeutic agents. Anti-TNF-α antibodies have been shown to improve anaemia in patients with rheumatoid arthritis [73,74], but to date no data are available on the use of these novel therapeutic agents in anaemic CKD patients.

Pentoxifylline is a phosphodiesterase inhibitor used in the management of peripheral vascular disease because of its anti-platelet effects and influences on erythrocyte deformability. Recently, anti-cytokine properties of this drug have been described including anti-TNF-α, anti-IFN-γ, anti-IL-10, anti-oxidant and anti-apoptotic effects [75,76]. Treatment with pentoxifylline induces a drop in TNF-α and IFN-γ production with a concomitant increase in Hb levels [75,76]. Once again, the potential of this agent in the management of anaemia of CKD has not been fully explored.

### Anaemia and co-morbidity—need for more study

To date, there has been a lack of well-designed interventional studies in CKD patients with inadequate response to ESA. With the exception of parenteral iron treatment for hyporesponsiveness to ESAs, most clinical studies conducted in this area have either been open and uncontrolled in design or have lacked the power to detect clinically important effects on ESA responsiveness.

However, before large and well-designed studies can be performed to investigate the therapeutic potential of some of the putative anti-inflammatory agents described in this review, more information regarding the characteristics of inflammatory hyporesponsiveness needs to be obtained. Low Hct levels and poor ESA responsiveness are often linked to inflammation, malnutrition and cardiovascular disease [5]. This triad constitutes the malnutrition–inflammation–atherosclerosis (MIA) syndrome and is strongly associated with a greater risk of death in haemodialysis patients [77]. Since persisting anaemia in ESA-treated patients is often associated with significant co-morbidity, increasing Hb levels without first attempting to treat underlying conditions may fail to reduce morbidity and mortality [78]. The results of future basic and clinical research into this intriguing area of clinical medicine are eagerly awaited.

### Conflict of interest statement
None declared.

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