



# The Anemia of Inflammation/Malignancy: Mechanisms and Management

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**Anemia is a common complication in patients with inflammatory diseases of many kinds, including cancer. The mechanisms that have captured the most attention include cytokine-mediated changes in both the production of and the response to erythropoietin (Epo), as well as important alterations in iron metabolism. The last is brought about by the relatively recently recognized peptide hormone, hepcidin. The availability of recombinant human Epo and its deriva-**

**tives (known by class as Erythropoietic Stimulating Agents, ESAs) has dramatically changed anemia management in patients with cancer but, in the process, has raised as many issues as have been answered. This chapter reviews the mechanisms resulting in anemia in inflammation, including cancer, and focuses on the controversies around management with the ESAs and the adjuvant use of iron in anemia management.**

A normocytic, normochromic anemia is common in patients with a variety of inflammatory disorders, including malignancy, and there are many contributing factors (Table 1). The most common form of anemia seen in patients with cancer or hematological malignancies results from the underproduction of red cells—a hypoproliferative anemia.<sup>1</sup>

Hypoproliferative anemias are characterized by a low reticulocyte production index and the absence of marrow erythroid hyperplasia despite significant, persistent anemia (hemoglobin < 10 g/dL). The mechanisms that lead to the anemia include impaired erythropoietin (Epo) production, an impaired response of the erythroid marrow to Epo, iron-restricted erythropoiesis (which itself impairs erythroid proliferation) and a diminished pool of Epo-responsive cells.

Generally, the chronic anemia associated with cancer is characterized by an inadequate production of Epo for a given hemoglobin/hematocrit as well as an inadequate response of the erythroid marrow to endogenous Epo.<sup>2</sup> In addition, there is impaired release of iron from stores as a result of increased hepcidin production<sup>3</sup> so that, in chronic conditions, there is evidence of inadequate delivery of iron to the erythroid marrow and evidence of iron-deficient erythropoiesis (termed functional or relative iron deficiency<sup>4</sup>). Finally, as with virtually all hypoproliferative anemias, there is a mild shortening of red cell survival. This constellation of findings is referred to as the anemia of chronic disease (ACD) or, more recently, the anemia of inflammation (AI).<sup>5-8</sup>

## Anemia as a Consequence of Cancer

The importance of anemia in the cancer patient has been studied extensively. Older studies used red cell transfusion needs as an indicator of significant anemia. As an example,

a frequently cited study by Skillings et al<sup>9</sup> indicated that in patients with malignancy, only about 19%, overall, received blood transfusions for anemia. However, 78% of patients with leukemia in that study required red cell transfusions. In patients with solid tumors, those with lung cancer had the highest frequency of transfusion. Of interest, patients with lung cancer were transfused at a relatively high hemoglobin level; this was attributed to their generally older age as well as the higher likelihood of concurrent pulmonary disease. Finally, there was a clear and stepwise correlation between the numbers of patients transfused when segregated by baseline hemoglobin level prior to chemotherapy. In that study, all 8 patients whose baseline hemoglobin was less than 8.0 g/dL required transfusion, while only 8% of the patients whose hemoglobin was  $\geq$  12.0 g/dL required transfusion.

These results can be compared to the transfusion re-

**Table 1. Mechanisms contributing to the anemia of inflammation/malignancy.**

1. Cytokine-mediated changes
  - Decreased erythropoietin (Epo) production
  - Decreased response of erythroid progenitors to Epo
  - Altered iron metabolism (relative iron deficiency)
2. Myelosuppressive effects of chemotherapy
  - Suppression of Epo production
  - Direct suppression of marrow function
3. Blood loss
4. Nutritional deficiencies
5. Hemolysis
  - Drug-induced
  - Microangiopathic
  - Autoimmune

quirements of patients in the U.S.-based multicenter trial of Epo treatment for patients with the anemia of cancer.<sup>10,11</sup> In the original studies, 28% of more than 300 patients with a variety of non-myeloid malignancies required transfusions over the 1 to 2 months prior to entering the study. In the first month of the study, before the full effect of Epo therapy was seen, the percentage of patients requiring transfusion rose to 44% in the group receiving cisplatin-based chemotherapy and remained at 26% in those patients receiving non-cisplatin-based therapy. Studies have shown that platinum-based drugs, in and of themselves, reduce Epo production by the kidney in a manner that correlates with drug-induced tubular dysfunction.<sup>12</sup>

### Changes in Red Cell Production Associated with Inflammation and Malignancy

The most common features of malignancy-associated anemia are the features of AI. These features include lower-than-expected circulating Epo levels in response to the anemia,<sup>2</sup> alterations in iron metabolism,<sup>8</sup> and a blunted erythroid progenitor cell response to Epo—all contributing to the hypoproliferative anemia.

The key to understanding the mechanisms surrounding these changes lies in the alterations in the production of several pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF $\alpha$ ), the interferons (IFN) and hepcidin. These cytokines are produced by monocytes, resident macrophages, T lymphocytes, bone marrow stromal cells, liver cells, and other tissues.<sup>8</sup>

### Epo Production

A useful system for the study of the regulation of Epo production has been hepatoma cell lines, such as HepG2 or Hep3B, which, when exposed to hypoxia, increase Epo production through transcriptional and translational mechanisms.<sup>13</sup> By using such a system, Faquin et al<sup>14</sup> showed that when Hep3B cells were exposed to hypoxia in the presence of IL-1, the expected increase in Epo mRNA expression and the release of immunoreactive Epo into the culture medium were blunted (**Figure 1**). Similar results were obtained with TNF $\alpha$ . This was not a toxic effect on the cells since the Hep3B cells responded to the combination of IL-1 and IL-6 by increasing Epo mRNA levels. The degree of suppression of Epo mRNA was dependent on the concentration of IL-1 added to the culture. These workers not only demonstrated cytokine concentration-dependent suppression of Epo mRNA accumulation but also provided a hierarchy of the effects of various cytokines with IL-1 $\beta$  being more suppressive than IL-1 $\alpha$  and TNF $\alpha$ . Jelkmann et al<sup>15</sup> had arrived at similar conclusions by demonstrating that IL-1 reduced Epo production by perfused rat kidneys.

One of the hallmarks of malignancy-associated anemia is the reduction in endogenous Epo levels with respect to the degree of anemia.<sup>2</sup> As shown in **Figure 2** (see Color

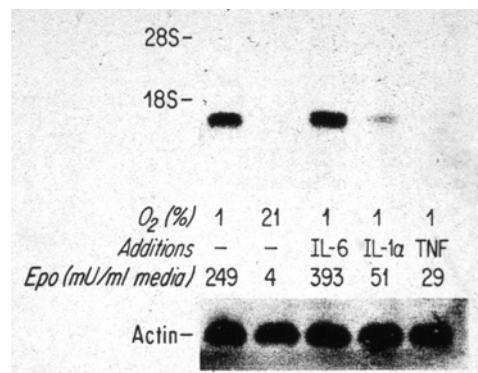
Figures, page 496), the expected inverse correlation between endogenous Epo levels, as measured by immunoassay, was lost in over 70 adult patients with malignancies of various kinds, including hematological malignancies. In contrast, patients with uncomplicated iron deficiency anemia showed an increase in circulating Epo levels that was inversely correlated with the decline in hemoglobin.

These laboratory and clinical results have led to the current belief that Epo production is blunted in adult patients with malignancy by the increased production of pro-inflammatory cytokines, which interfere with the hypoxia-induced upregulation of Epo gene expression. This may not be true in all circumstances since at least one report indicates that an impaired response to endogenous Epo rather than impaired Epo production may characterize cancer-related anemia in children.<sup>16</sup>

### Erythroid Marrow Response to Epo

Much has been learned about the effect of various inflammatory cytokines on the response of the erythroid marrow to Epo. These studies have been carried out both in vitro and in vivo and have identified a number of interacting mechanisms which likely contribute to the hypoproliferative nature of AI.

Some of the earliest studies employed bone marrow cells aspirated from anemic patients with infectious diseases such as tuberculosis or histoplasmosis. When bone marrow adherent cells were separated from non-adherent cells, and the non-adherent cells placed into semisolid medium with Epo, there was a marked increase in the num-



**Figure 1. Effect of IL-6, IL-1 $\alpha$ , and TNF- $\alpha$  on hypoxia-induced erythropoietin (Epo) mRNA levels.** Hep3B cells were incubated under normoxic (21% O<sub>2</sub>) or hypoxic (1% O<sub>2</sub>) conditions in the presence or absence of the various cytokines, as indicated. Actin served as control. In addition, the amount of Epo produced by the cells was determined in duplicate by RIA as shown.

Reproduced with permission from Faquin et al. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. *Blood* 1992;79:1987-1994. © American Society of Hematology

ber of erythroid colonies (from erythroid colony-forming cells [CFU-E]) observed when compared to cultures of unfractionated bone marrow cells.<sup>17</sup> When these same adherent cells (most likely macrophages) were added back to the culture of non-adherent cells, erythroid colony growth was suppressed in a cell dose-dependent manner. Of significance, when adherent cells were separated from the marrow of non-anemic patients who had inflammatory/malignant disease, there was no increase in erythroid colony growth, and when the adherent cells were recombined in culture with the non-adherent cells, no suppression of colony growth was observed.

Findings similar to these were reported by Roodman et al.<sup>18</sup> These investigators found that the removal of adherent cells from total bone marrow resulted in increased erythroid colony formation if the marrow source was a patient with anemia and chronic inflammatory disease or malignancy. Unlike the studies of Zanjani et al,<sup>17</sup> however, there was no allogeneic inhibitory effect seen when the adherent cells from anemic patients were placed into co-culture with non-adherent cells from non-anemic subjects. Also, medium conditioned by adherent cells from anemic patients was inconsistent in its ability to suppress normal erythroid colony formation. Nevertheless, this set the stage for the identification of soluble factors that were released by monocytes/macrophages under conditions of inflammation.

Two lines of investigation have provided insights into the process. First, in vitro studies by Broxmeyer et al<sup>19</sup> and Means<sup>7</sup> demonstrated the complexity of the factors at work. In earlier studies, the latter group showed that the addition of various cytokines to cultures of either human or murine bone marrow cells resulted in a concentration-dependent suppression of erythroid colony formation.<sup>20-23</sup> The effect is likely due to the pro-apoptotic action of the cytokines on the cell. The most sensitive progenitor cells under these conditions were CFU-E as opposed to the more primitive erythroid burst-forming cells (BFU-E). That this was not a toxic effect of certain cytokines on the progenitor cells was shown by the restoration of full erythroid colony growth by the addition of high concentrations of Epo to the cultures.

Similarly, Johnson et al<sup>24</sup> demonstrated that the in vivo administration of IL-1 to mice resulted in a selective suppression of the numbers of CFU-E in bone marrow and spleen. Again, this suppression could be overcome by the nearly simultaneous administration of Epo to the animals. Of interest, 48 hours after IL-1 administration, there was a marked increase in the numbers of splenic and marrow BFU-E, granulocyte/macrophage colony-forming cells (GM-CFC), G-CFC and megakaryocytic (Meg)-CFC.

Johnson and co-workers<sup>25</sup> made similar observations in mice chronically exposed to TNF. In these studies, nude mice were injected with Chinese hamster ovary cells that had been transfected with the human TNF gene. These cells

produced high levels of TNF constitutively. In these animals, there was a profound suppression of the numbers of marrow and splenic BFU-E and CFU-E. In contrast, marrow and splenic GM-CFC and mixed-cell CFC were not affected.

Means and Krantz provided an even more refined definition of the cytokine networks that may be involved in AI. Through a series of experiments beginning with unfractionated human marrow cells, these investigators showed that for IL-1 to suppress erythroid colony formation, accessory cells had to be present, and it was the accessory cells that released gamma interferon ( $\gamma$ IFN) in response to IL-1.<sup>26</sup> This suppression of in vitro erythropoiesis was reversible by the addition of high concentrations of Epo to the culture system. In contrast, TNF was shown to suppress in vitro erythropoiesis by stimulating the release of  $\beta$  IFN from marrow stromal cells. This suppressive effect could not be overcome by exogenous Epo. Similarly,  $\alpha$  IFN was shown to not suppress erythropoiesis directly, but, rather, to stimulate the release of an as yet to be defined molecule from a subset of T lymphocytes. This effect also was not reversible by Epo.<sup>26</sup> Finally, Dallaglio et al have reported that hepcidin, itself, can inhibit erythroid colony formation in culture in the presence of low Epo concentrations.<sup>27</sup>

The results of the in vitro and preclinical animal studies found support in the studies of Papadaki et al.<sup>28,29</sup> In two related reports, these investigators demonstrated that locally produced TNF $\alpha$  was associated with reduced numbers of erythroid progenitors in the bone marrow of patients with active rheumatoid arthritis, and that long-term marrow cultures from these patients failed to support the generation of CFC. The mechanism for the loss of erythroid cells was increased apoptosis. When follow-up studies were carried out, either in vitro or after the patients were treated with infliximab, an antibody against TNF $\alpha$ , many of the cellular defects were reversed, and the patients at least partially corrected their anemia. TNF $\alpha$  is only one of the cytokines involved in the complex networks of response to inflammation and tissue injury in man. Thus, the presence in increased concentrations of these cytokines, either systemically or locally, can have a clinically significant effect on erythropoiesis.

These findings, taken together, demonstrated a specific cytokine-mediated suppression of erythropoiesis that was reversible by Epo in some instances, but not all. As these studies are examined retrospectively, it becomes clear why the doses of recombinant human Epo required to correct the anemia of malignancy are higher than the Epo doses required to correct the anemia of chronic renal failure. These findings also may explain why a subset of patients with malignancy-associated anemia did not respond to the doses of recombinant human Epo that were employed in the initial clinical trials.

## Changes in Iron Metabolism

Characteristic of AI is a reduction in the serum iron level. This is mediated by hepcidin, the 25–amino acid polypeptide hormone that is central to iron trafficking. In the main, hepcidin is released by the liver and circulates to interact with its cellular receptor, the iron export channel ferroportin, to block release of iron from cells such as tissue macrophages and jejunal enterocytes.<sup>30</sup>

However, hepcidin is also produced in murine and human monocytes. In murine monocytes, hepcidin production is increased in response to bacterial pathogens in a toll-like receptor 4 (TLR-4)–dependent manner.<sup>31</sup> This pathway is distinct from the pathway regulating hepcidin production in the murine liver. Hepcidin production is upregulated in human monocytes by IL-6 and lipopolysaccharide and can act in an autocrine fashion to downregulate ferroportin expression and, thereby, iron export from storage cells.<sup>32</sup>

The net effect of hepcidin, whether acting via an autocrine mechanism or through the circulation, is trapping of the iron within iron-storage cells and a block in the absorption of iron from the gut. This leads to a reduction in the serum iron level and the percent of transferrin saturation. As a result, there is iron-restricted erythropoiesis and, if severe, even the production of microcytic, hypochromic red cells. The corollary to this is a gradual buildup of iron in cells of the reticuloendothelial system and a rise in the serum ferritin. The increase in the serum ferritin actually has two components—reflecting the increase in iron stores and in response to the general inflammatory process, itself, since ferritin is an acute phase reactant. This was shown nicely by Elin et al<sup>33</sup> a number of years ago. By giving a single injection of bacterial endotoxin or the fever-inducing steroid etiocholanolone to normal volunteers, these workers demonstrated a rapid fall in serum iron and a rise in serum ferritin. The effects lasted for up to 10 days.

An exception to the more common clinical picture may be cases of inflammation in children, where the demand for iron to support growth results in true iron deficiency. The anemia can respond to exogenous iron despite ongoing inflammation. This typifies systemic-onset juvenile chronic arthritis, which is characterized by high levels of circulating IL-6, reduced marrow iron stores, appropriate levels of circulating Epo, and correction of the anemia with iron supplementation.<sup>34</sup> The authors of this study appropriately concluded that the clinical phenotype of AI is likely due to the type of cytokine(s) released in response to the inflammation.<sup>34</sup>

In the liver, hepcidin gene expression is regulated through at least two pathways. One pathway is dependent on iron availability and involves signaling from the surface of the hepatocyte through the BMP receptor complex.<sup>35</sup> This signaling complex also involves proteins such as hemojuvelin that, when mutated, are associated with iron overload syndromes in humans such as juvenile hemochromatosis. It is likely this pathway that also involves *Hfe*, the

gene most commonly mutated in hereditary hemochromatosis, and *TfR-2* (transferrin receptor-2).

A second well-recognized pathway that regulates hepcidin gene expression is the IL-6–mediated inflammatory signaling pathway.<sup>36,37</sup> IL-6 directly upregulates hepcidin gene expression. Both of these pathways, the iron stores/availability and IL-6-mediated inflammatory response pathways, come together through Smad 4.<sup>35</sup>

More recently, and pertinent to the use of erythropoietic-stimulating agents (ESAs) to treat AI, are reports of the administration of Epo downregulating hepcidin gene expression in the livers of intact mice.<sup>30</sup> Hypoxia and anemia in intact animals are known to downregulate hepcidin. When Epo was injected into C57BL/6 mice for 3 days, Northern blot analysis of the livers for hepcidin mRNA showed almost no transcripts 24 hours after the last Epo injection. Similarly, Pinto et al<sup>38</sup> have recently reported that Epo mediates hepcidin gene expression in isolated murine hepatocytes and the human hepatoma cell line HepG2 through Epo receptor signaling and involving the transcription factor *C/EBP $\alpha$* .

## Treatment of AI/Malignancy

Whenever possible, the best approach to the treatment of AI is effective treatment of the underlying condition. However, that is often not possible, particularly with chronic diseases that are not always curable, such as chronic renal failure in patients unable to be transplanted or patients with incurable cancers. In the latter instance, patients who undergo chemotherapy are particularly vulnerable, and it is estimated that more than 50% will require one or more red cell transfusions before they finish treatment. ESAs have been available for treatment of chemotherapy-induced anemia for 15 years, and their use has changed the landscape for such patients.

Early studies with ESAs focused on transfusion avoidance and that was the clinical endpoint for which the drug was approved. The target hemoglobin was usually in the 10 to 11 g/dL range, and the FDA-approved package insert reflected that. Follow-on, open label and community-based trials focused on quality of life, and a number of them demonstrated that, with increasing hemoglobin, there was a continued improvement in quality of life, with the largest reported increment coming between 11 and 12 g/dL.<sup>39</sup> However, the one U.S. trial that was randomized and blinded demonstrated an increase in hemoglobin and a reduction in transfusion requirements, but failed to reach statistical significance for improvement in quality of life.<sup>40</sup> In contrast, the trial by Littlewood et al,<sup>41</sup> which was also a randomized double-blinded trial, did show a significant improvement in quality of life for those patients receiving the ESA. That study also had the hopeful outcome suggesting improved patient survival, although the outcome was not statistically significant ( $P = .13$ ; log rank test).

This set the stage for other trials that had survival or disease progression as the primary endpoints, were adequately powered, and involved patients with specific tumor types. Higher doses of ESA were used to raise the hemoglobin to near-normal levels. The first trial, in head and neck cancer, tested the hypothesis that by normalizing the hemoglobin, better tissue oxygenation would be achieved and tumors would be more susceptible to radiation therapy. In fact, the outcome was the opposite, with poorer loco-regional control and poorer progression-free survival and overall survival.<sup>42</sup> While this study had many problems (not the least of which was the extremely high hemoglobin achieved in some patients), the outcome focused attention on other trials that were already underway. As the results of the other trials became available, concerns for the safety of ESAs in treating the anemia of cancer and cancer chemotherapy grew. Either poorer survival or shorter progression-free survival times have now been reported in a number of trials using ESAs in head and neck cancer, lung cancer<sup>43</sup> and breast cancer<sup>44</sup> (and summarized by Bennett et al<sup>45</sup>). This has led to the current FDA-mandated 'black box' warnings on the package inserts for all members of the ESA class.

At the same time that these trials were being reviewed, other trials were underway to look at the effect of supplemental parenteral iron on the success of achieving a hematologic response (defined as a  $\geq 2$  g/dL increase in hemoglobin over a 16-week period or reaching a hemoglobin of 12 g/dL). The use of parenteral iron has been shown repeatedly to increase hemoglobin concentrations while reducing ESA dose requirements in patients with chronic kidney disease. The first to convincingly show the positive effect of parenteral iron in cancer patients receiving chemotherapy was Auerbach et al.<sup>46</sup> However, the population chosen included many patients with true iron deficiency anemia. Henry et al<sup>47</sup> reported similar positive results. Recently, three European studies have appeared that clearly demonstrated the beneficial effect of providing supplemental parenteral iron while giving ESAs to patients with indolent lymphoproliferative disorders<sup>48</sup> or in the setting of chemotherapy-associated anemia.<sup>49,50</sup> In each case, the target hemoglobin was achieved in a greater percentage of patients and in a shorter period of time in those receiving iron versus those in the control arm (**Figure 3**; see Color Figures, page 496). The last two studies were published simultaneously along with an editorial by Auerbach<sup>51</sup> suggesting that intravenous iron should become a routine part of the management of chemotherapy-induced anemia in cancer patients.

There is no doubt that providing additional iron parenterally enhances the response to ESAs in a variety of settings in which AI needs treatment, whether or not associated with chemotherapy. But it doesn't answer the critical question of whether reducing the dose of ESA needed to achieve a certain hemoglobin target will eliminate the risks, both real and perceived, associated with the use of

these agents.<sup>45</sup> As a result, with so many recent restrictions in the use of the ESAs for managing anemia in the chemotherapy-treated cancer patient, and without knowing whether there are long-term adverse effects of the use of parenteral iron in these patients, it is premature to promote guidelines.

At the present time, the FDA recommends restricting the use of ESAs to patients who are receiving chemotherapy, whose hemoglobin falls below 10 g/dL (and who, therefore, might require red cell transfusions) and who are not being treated with curative intent. What remains to be determined is whether or not the use of any ESA versus transfusion support in this patient population leads to different outcomes.

### Disclosures

Conflict-of-interest disclosure: The author declares no competing financial interests.

Off-label drug use: Erythropoietin regulates red blood cell production. It is used to combat anemia in a number of clinic situations. It is also used widely for non-approved indications such as MDS. Of interest, it also may have non-erythropoietic effects that need to be tested clinically.

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