Pathogenesis and treatment of anemia in inflammatory bowel disease

Günter Weiss[1] and Christoph Gasche[2]

[1]Department of Internal Medicine I, Clinical Immunology and Infectious Diseases, Medical University of Innsbruck, Austria, [2]Department of Medicine III, Division of Gastroenterology and Hepatology and Christian Doppler Laboratory for Molecular Cancer Chemoprevention, Medical University of Vienna, Austria. E-mail: guenter.weiss@i-med.ac.at. *doi:10.3324/haematol.2009.017046*

Anemia in inflammatory bowel disease (IBD) is a prototype of a combination of iron deficiency and anemia of inflammation (*i.e.* anemia of chronic disease; ACD)[1] which is caused by negative effects of an activated immune system at different levels of erythropoiesis. Besides iron deficiency and ACD, metabolic disturbances and vitamin deficiencies as well as commonly used IBD drugs can aggravate anemia in IBD.[2,3]

Pathophysiology

Inflammation affects three major steps essential for normal erythropoiesis and can, therefore, lead to the development of anemia of inflammation. These effects are: (i) an immunity-driven diversion of iron traffic leading to retention of the metal in macrophages and thus to iron-deficient erythropoiesis; (ii) blunting of the biological activity of erythropoietin, the major erythropoiesis-stimulating hormone; and (iii) inhibition of the differentiation and proliferation of erythroid progenitor cells.

The changes in body iron distribution are caused by cytokines and acute phase proteins. Among these, the master regulator of iron homeostasis, hepcidin, appears to play a pivotal role. Hepcidin is an anti-microbial peptide formed of 25 amino acids, which is produced mainly in the liver in response to iron overload or upon induction by pro-inflammatory stimuli, such as lipopolysaccharide or interleukin-6. Hepdicin exerts its biological function upon binding to the only known cellular iron exporter, ferroportin (SLC40A1), thereby leading to ferroportin internalization and degradation.[4] This blocks the transfer of absorbed iron from the duodenal enterocyte into the circulation – an effect which can be aggravated by tumor necrosis factor (TNF)-α[5] – and, in parallel, causes retention of iron within macrophages and monocytes.[6] The latter effect is of major importance, because macrophages are involved in the re-utilization of iron from senescent red blood cells which are taken up via erythrophagocytosis. Within the phagocytes, iron is recycled through enzymatic degradation of heme and delivered via ferroportin into the circulation, accounting for approximately 90% of the daily iron needs.[7] During inflammatory processes, however, the biological half-life of erythrocytes is reduced as a consequence of oxidative stress and lipid-peroxidation, thus promoting erythrophagocytosis and reducing iron recirculation.[1,7] In addition, pro-and anti-inflammatory cytokines further contribute to iron retention within monocytes/macrophages by stimulating iron uptake pathways while inhibiting ferroportin transcription in these cells.[8] All these events lead to retention of iron within phagocytes and to the development of functional iron deficiency, meaning that although iron is abundant in the body the metal is not available for erythropoiesis.

In IBD, this scenario is typically associated with iron deficiency which is a consequence of recurrent bleeding episodes from ulcerated intestinal mucosa. The combination of functional iron deficiency due to ACD and chronic blood loss is the most common finding in IBD. In fact, whenever there is intestinal inflammation (*i.e.* mucosal ulceration), parallel blood loss (through the ulcerated mucosa) must be expected. Therefore, IBD-associated anemia is the prototype of iron deficiency combined with ACD. Chronic bleeding studies have also indicated reduced iron absorption or dietary iron restriction.[9]

Erythropoietin levels in ACD have been found to be inadequate for the degree of anemia in many but not all conditions including IBD[9,10] which may be partly due to interleukin-1- and TNF-α-mediated inhibition of erythropoietin promoter activity and a cytokine-driven formation of toxic radicals, thereby damaging erythropoietin-producing cells and inhibiting erythropoietin formation in the kidney.[11] Moreover, cytokines can interfere with erythropoietin/erythropoietin receptor-mediated signaling which involves the signal transducer and activator of transcription (STAT) family and mitogen and tyrosine kinase phosphorylation pathways. Accordingly, in the presence of circulating interferon-γ or TNF-α, much higher amounts of erythropoietin are needed to restore the formation of erythroid colony-forming units (CFU-E) in the bone marrow.[12] In addition, the immunity-driven down-regulation of erythropoietin receptors on erythroid progenitor cells and the limited availability of iron for heme biosynthesis negatively affect the biological functions of erythropoetin.[1,10]

Pro-inflammatory cytokines, such as interferon-γ, interferon-α, TNF-α and interleukin-1, inhibit erythopoiesis by exerting pro-apoptotic effects towards erythroid burst-forming units (BFU-E) and CFU-E.[1,12] In addition to the limited availability of iron for erythropoiesis, the cytokine-mediated down-regulation of erythropoietin-receptor expression on progenitor cells, impaired biological activity of erythropoietin, reduced expression of other hematopoietic growth factors such as stem cell factor as well as toxic effects of radicals such as nitric oxide or superoxide anion further inhibit erythroid progenitor cell proliferation.[1,12] The acute phase protein α-1 antitrypsin effectively binds to transferrin receptor and inhibits the iron uptake into erythroid progenitors mediated by this receptor, thus blocking the cells' growth and differentiation. The iron storage protein ferritin has been described to have anti-proliferative effects, which may be due to a limitation of iron availability for progenitor cells.[1]

Finally, anemia in IBD can also be induced by deficiency of vitamins, such as cobalamin and folic acid, a condition which further impairs the proliferation of hematopoietic progenitor cells,[13] or by certain medications, such as thiopurine analogs (6-mercaptopurine, azathioprine), sulfasalazine and methotrexate, most of which can inhibit erythropoiesis directly.

Diagnosis

The severity of anemia in IBD varies considerably.[9] An international working party recently published guidelines on the diagnosis and treatment of anemia in IBD.[14] The lower hemoglobin levels below which anemia was defined as present were those proposed by the World Health Organization (non-pregnant women, 12.0 g/dL; men 13.0 g/dL). Most patients with IBD have mild to moderate anemia (hemoglobin above 10.0g/dL), but in the presence of bleeding episodes, the hemoglobin concentration may decrease further.[2,3] In order to provide the appropriate treatment, it is essential to distinguish between predominantly iron deficiency anemia (IDA) and iron deficiency with ACD (ACD/IDA).[15] ACD without iron deficiency is uncommon and only seen after excessive intravenous iron replacement therapy.[16] It is characterized by typical changes of body iron homeostasis.[1,17] While circulating concentrations of the iron storage protein ferritin are low (<30 μg/L) in patients with IDA, they are normal or increased in patients with ACD. This is due to two factors. One is that the increased ferritin levels reflect iron retention within monocytes and macrophages, the other is that ferritin expression is induced by inflammation and, therefore, ferritin levels do not exactly reflect the amount of stored iron in patients with IBD unlike the situation in subjects without inflammation.[17] This is the reason why the international guidelines recommend using 100 μg/L as the lower ferritin cut-off in active IBD.[14]

Because serum iron concentrations and transferrin saturation, as well as zinc protoporphyrin levels, are low in IDA, ACD and ACD/IDA, these parameters cannot help in the differential diagnosis. In contrast, the concentration of transferrin moves in the opposite direction.[6] While being normal or low in ACD, it is increased in IDA. The soluble transferrin receptor is a truncated fragment of the membrane receptor, and soluble transferrin receptor levels are increased when the availability of iron for erythropoiesis is low, as in IDA.[1,17] The concentration of soluble transferrin receptor and the ratio of log ferritin/soluble transferrin receptor have been found to be helpful in the differential diagnosis between ACD and ACD/IDA[15,17] but published data exist only for pediatric IBD.[18] While ACD is mostly normochromic and normocytic, ACD/IDA more frequently presents as microcytic and hypochromic anemia.[6,15] Other aids to the differentiation between the two conditions include quantification of reticulocyte hemoglobin and the percentage of hypochromic red cells, which indicate the availability of iron for erythroid progenitors, as well as determination of hepcidin in serum.[15,17] Future studies should focus on identifying parameters that reflect ion deficiency in the state of active IBD.

Treatment

The persistence of anemia is associated with impaired cardiac and renal function, reduced systemic oxygen delivery, decreased physical activity, fatigue, and impaired quality of life.[2,3,19] When possible, the best therapeutic approach to ACD is relief of the underlying disease. However, if this cannot be achieved or when the anemia is severe, specific anemia treatment is mandatory. It is clear that iron must be provided in cases of IDA.

Blood transfusions are widely used as an immediate intervention for rapid correction of severe or life-threatening anemia. However, such transfusions do not correct the

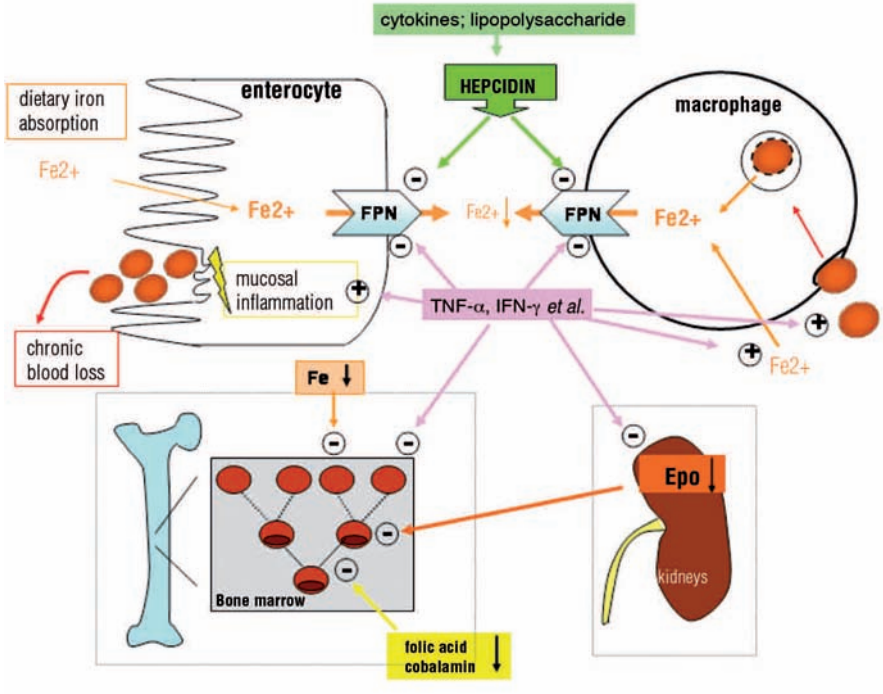

Figure 1. Pathophysiological mechanisms contributing to anemia in patients with IBD. Cytokines induce the formation of hepcidin which then blocks cellular iron (Fe^{2+}) export via ferroportin (FPN) from macrophages and inhibits the transfer of absorbed iron from enterocytes to the circulation. This results in a reduction of circulating iron and limited availability of the metal for erythroid progenitors, thus blocking erythropoiesis. Cytokines, such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ, also aggravate iron restriction in macrophages by stimulating iron uptake and promoting erythrophagocytosis while inhibiting iron export via repression of ferroportin transcription. In addition, TNF-α further contributes to blockage of iron absorption. Iron deficiency can also be aggravated by chronic blood loss from intestinal lesions caused by active IBD. Erythropoiesis is further impaired as a consequence of a cytokine-mediated reduction of the formation and/or the biological activity of erythropoeitin (Epo) along with direct anti-proliferative effects of these cytokines on erythroid progenitor cells. Finally, vitamin deficiencies can impair erythroid differentiation.

underlying pathology and do not have a lasting effect. The decision on whether to administer blood should not, therefore, be based only on the hemoglobin level, but should also take clinical symptoms and co-morbidity into account. Whether blood transfusions affect immune function and whether they are cause-effectively linked to mortality in patients undergoing surgery[20] or being treated in intensive care units remains controversial.[21]

Iron supplementation has been shown to be an effective therapy in IBD patients with anemia.[22] Administration of iron is most effective, and mandatory, in subjects with ACD/IDA since these individuals are completely depleted of iron. The presence of ACD should not, therefore, prevent the administration of iron in this population of patients. Oral iron is, however, poorly absorbed because of the inhibition of iron absorption in the duodenum as a result of the combined actions of hepcidin and TNF-α,[4,5,15] which was nicely demonstrated in a clinical study of patients suffering from anemia and IBD.[23] For this reason, the intravenous route of iron administration is preferable and recommended,[14] particularly in patients with ACD/IDA receiving erythropoeisis-stimulating agents (ESA). Low transferrin or erythropoietin levels are good predictors of the need for ESA therapy.[24] Most studies in this setting are on iron sucrose, but the new iron carboxymaltose has also been proven to be highly effective in a large multicenter trial.[25,26]

Human erythropoietin derivatives and ESA have been found to be effective in IBD patients suffering from ACD/IDA, specifically when large amounts of intravenous iron were administered without significant success.[16,22] ESA therapy is intended to overcome the negative effects of an activated immune system on the biological activity of endogenous erythropoietin and to stimulate BFU-E/CFU-E proliferation; however, the mechanism of action of ESA in ACD is still largely elusive.[1]

In this issue of the Journal, Bergamaschi *et al.* present a careful analysis of anemia in patients with IBD.[27] In an assessment of 263 out-patients with IBD, they observed that almost two-thirds of their patients were anemic at diagnosis and that the relative percentage was slightly higher in subjects with Crohn's disease than in patients suffering from ulcerative colitis. Treatment of IBD gradually increased hemoglobin levels over time but 35% of all patients remained anemic, indicating that anemia is a frequent and so far underestimated complication in patients with IBD. The presence of anemia was positively associated with disease activity, confirming that an activated immune system and disease-associated lesions of the gut are the major contributors to anemia in IBD. This was confirmed by a subsequent analysis by the authors, which indicated that the most frequent pathologies underlying anemia in IBD were ACD, IDA and ACD/IDA followed by vitamin deficiencies, while some anemias were multifactorial or could not be clearly classified. Surprisingly, ACD was most prevalent at diagnosis and the frequency decreased with IBD treatment while IDA was less frequent at the initial presentation, but its relative contribution increased significantly as the patients were followed-up. This may indicate that, at diagnosis, most patients suffer from inflammation-driven ACD. During follow-up true iron deficiency, as a consequence of chronic intestinal bleeding, becomes more important whereas immune system-driven processes, likewise as a consequence of immunosuppressive IBD treatment, contribute less to the pathogenesis of anemia. Accordingly, patients with ACD had higher serum levels of the hepcidin precursor pro-hepcidin compared to IDA subjects and increased pro-hepcidin levels were associated with elevated ferritin concentrations, supporting a role of hepcidin in the pathogenesis of IBD-associated anemia and nicely resembling observations made in rheumatic and infectious diseases.[6]

Most interestingly, Bergamaschi *et al.*[27] present data on the therapeutic effects of anti-TNF-α treatment with infliximab on the resolution of anemia in a subgroup of patients with Crohn's disease. They found that patients who responded to treatment started to have an improvement in their anemia within 2 weeks after the first infusion of infliximab. This was paralleled by a significant improvement of the Crohn's disease activity index. Patients responding to therapy were found to have an increase of endogenous erythropoietin levels over time while ferritin levels decreased, which may indicate iron mobilization as also reflected by a slight increase of transferrin saturation. The data suggest that infliximab neutralizes the inhibitory effects of TNF-α on erythropoietin production,[11] and increases the availability of iron for erythropoiesis. Mechanistically, this may be explained by reduced cytokine-induced formation of ferritin and hepcidin and subsequent improvement of intestinal iron absorption and iron release from macrophages via ferroportin-mediated iron export.[4,5,8] *In vitro*, infliximab improved the proliferation of cultured BFU-E which relates to blockage of inhibitory effects of cytokines on erythroid progenitor cells.[12] On the other hand, infliximab also induces mucosal healing thereby reducing the amount of pro-inflammatory cytokines produced and, even more importantly, the amount of blood lost through mucosal ulcers.

We certainly need more detailed information on the underlying mechanisms by which infliximab improves anemia in many, but not all, IBD patients, but we should also consider whether other therapies may be effective. For example, since interleukin-6 is the major inflammation-driven inducer of hepcidin identified so far,[4] therapy with anti-interleukin-6 may be an even more promising approach for resolving anemia in IBD patients. New therapeutic strategies may also emerge from our expanding knowledge on the pathophysiology of ACD and the underlying regulatory circuits of iron homeostasis. Such new therapies may include the neutralization of hepcidin to overcome the retention of iron within moncoytes/mancrophages, modifiers of erythropoietin and/or erythropoietin receptor sensitivity, and new hormones and cytokines/anti-cytokines which can effectively stimulate erythropoiesis and/or counterbalance iron restriction under inflammatory conditions.

This leads to the final point, namely the definition of therapeutic end-points for anemic IBD patients in whom anemia does not resolve or recurs fast. No data from prospective clinical trials are available to help with this issue. One would assume that complete resolution of anemia should be the expected therapeutic end-point. A recent outcome study looked into the follow-up after successful therapy of anemia and found fast recurrence of

iron deficiency (and anemia) in patients with IBD.[28] Half of all patients who had received intravenous iron therapy (with or without ESA) relapsed within 10 months after therapy. These data point to the need for maintenance treatment (with iron and possibly also ESA). A prospective, placebo-controlled clinical trial (FERGI) is currently underway and its results are expected at the end of 2010. Large outcome studies are also needed to determine whether the resolution of anemia actually improves the clinical course of IBD.

Guenter Weiss, MD is Professor of Medicine at the Medical University of Innsbruck and heads the Division of Clinical Immunology and Infectious Diseases at the Department of Internal Medicine I. His research focuses on disorders of iron homeostasis with a major emphasis on the anemia of chronic disease, iron overload syndromes, host-pathogen interactions and innate immunity.

Christoph Gasche, M.D. is a gastroenterologist with an interest in inflammatory bowel diseases and mechanisms of inflammation-associated colorectal carcinogenesis. He is currently Director of the Christian Doppler Laboratory for Molecular Cancer Chemoprevention (www.meduniwien.ac.at/innere3/gaschelab) and Associate Professor of Medicine at the Division of Gastroenterology and Hepatology at the Medical University of Vienna, Austria.

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