The anaemia of infection

Jack Metz

It is a great privilege to contribute to the Festschrift in honour of Professor Hendrik Koornhof. Internationally acclaimed researcher, academic and teacher, he has been the mentor of a whole generation of medical microbiologists. But above all, he has set an example as a humanitarian, rarely equalled by his peers. His numerous acts of kindness and concern for others, especially the underprivileged, are legendary. A man of the highest ethical and moral principles, he has always been intolerant of injustice, and has never hesitated to speak out against it, irrespective of the personal cost. He has enriched the lives of all of us who have been fortunate to know him.

Professor Koornhof's major research contributions are in the field of bacterial infectious disease, to which he has made numerous seminal contributions. It seemed fitting then to devote this paper to the anaemia of bacterial infection.

Anaemia is a cardinal feature in patients with bacterial infections, particularly infections lasting longer than a month. It occurs with a wide spectrum of infections, especially tuberculosis, chronic pyogenic infections, osteomyelitis, pneumonia, subacute bacterial endocarditis, pulmonary abscesses, empyema, cellulitis and chronic urinary tract infection.[1] Anaemia is present in most patients with active pulmonary tuberculosis.[2] The anaemia occurs as a result of immune stimulation, and is common to all inflammatory disease. Originally termed the 'anaemia of infection', it was subsequently included within the 'anaemia of chronic disease', and is now regarded as an example of the 'anaemia of inflammation'. For more detailed information on the anaemia of infection and the anaemia of chronic disease, the reader is referred to two recent comprehensive reviews.[3,4]

Pathogenesis

The anaemia of infection (AI) is probably an essential protective response to chronic infection. It reflects activation of the immune system designed to withhold iron from invading pathogens. Bacteria require iron as an essential element for survival and normal growth, due to its role in mitochondrial respiration. The AI is mediated by cytokines, the action of which withholds iron from the marrow, directly depresses erythropoiesis and damages red cells, all of which result in anaemia. A further factor could be erythrophagocytosis, but this is unlikely to contribute significantly to the anaemia, except perhaps in the haemophagocytic syndrome.

The role of cytokines in the anaemia of infection

Cytokines produced as mediators of the host immune response to infection are responsible for the development of the AI. Cytokines are proteins released by one cell population on contact with specific antigen, and act as intercellular mediators in the generation of an immune response. The invasion of bacteria leads to activation of T-lymphocytes and macrophages, which induce the production of the cytokines interferon-gamma (INF-γ), tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6). IL-6 stimulates the hepatic synthesis of hepcidin,[5,6] the effect of which is to divert iron into stores rather than release, resulting in iron-restricted erythropoiesis and anaemia.

Cytokines also impair red cell production in the marrow. IL-1 and TNF-α inhibit the production of erythropoietin (Epo), and together with INF-γ impair responsiveness of progenitor cells to Epo.[7] An inhibitory effect of TNF-α on red cell production in the bone marrow has been demonstrated by both *in vitro* and *in vivo* studies. INF-γ directly suppresses the proliferation of erythroid progenitor cells. In this way, cytokines impair the physiological Epo response to the anaemia. In addition, TNF-α directly damages erythrocytes and decreases red cell life span.

In infection, cytokines and their products therefore cause: (*i*) a diversion of iron into iron stores in the RES, resulting in decreased iron concentration in the plasma, thus limiting its availability to red cells for haemoglobin synthesis; (*ii*) an inhibition of erythroid progenitor cell proliferation; and (*iii*) inappropriate production and activity of Epo.[8] The first leads to anaemia and the latter two result in suboptimal response of the bone marrow to the anaemia. AI is therefore basically an underproduction anaemia due to iron restriction, combined with inability of erythropoiesis to compensate adequately for the anaemia. Reduced red cell survival is an additional, but less important factor in the genesis of the anaemia. The cytokine-

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induced reduction in the availability of iron together with the decreased production of and response to Epo are the major factors in the development of AI.

Iron metabolism in infection

Humans have developed defence mechanisms to withhold iron from bacteria, which grow faster when iron is abundant. Infections induce iron sequestration in macrophages, and also decrease iron absorption from the small intestine. This probably limits iron availability to invading bacteria, thereby containing the infection, and as such is part of the body's immune mechanism.[9] This diversion of iron to stores is due to the action of cytokines on the iron-regulating peptide, hepcidin. Hepcidin is synthesised in the liver, released into the plasma and excreted in the urine.[10] It is the dominant regulator of the absorption of dietary iron, its release from macrophages, and mobilisation from hepatic stores.

Hepcidin synthesis is rapidly induced by the cytokine IL-6, which is produced at the site of infection. Hepcidin concentration increases in response to infection, retaining iron in macrophages that would otherwise be released into plasma, and decreasing plasma iron concentration. Unfortunately, this diversion of iron from the circulation into stores limits the availability of iron for erythroid progenitor cells and results in iron-restricted erythropoiesis and the development of anaemia.

The bone marrow in infection

In anaemia, there is usually an increased production of Epo to stimulate bone marrow production of red cells to compensate for the anaemia, and the Epo response is commensurate with the degree of anaemia. This is not the case in the AI, where the Epo response is inadequate for the degree of anaemia, as has been reported in tuberculosis.[11] This is a cytokine-mediated effect, as described above. It is not clear which of the two factors, limitation of the iron supply or inadequate Epo, is the major rate-limiting factor in the pathogenesis of the AI.

Whereas the diversion of iron from the plasma can be seen as a defence mechanism to deprive iron from the invading bacteria, the reason for the reduced Epo production and response of erythropoiesis to Epo that is associated with infection is not clear. One possible explanation is that this action at the marrow level limits the deleterious effect of the iron deprivation to erythropoiesis, which is set at a lower level, more commensurate with the available iron supply. This may explain why iron deficient anaemia does not develop in all infected subjects, and why the AI is less often microcytic/hypochromic than normocytic/normochromic. It could be argued that with a normal level of erythropoiesis in the marrow, the anaemia could not be corrected in the absence of an adequate supply of iron.

Red cell life span in infection

The moderate shortening of the red cell life span in AI due to the damage inflicted on the red cells by increased TNF-α is not regarded as a major factor in the pathogenesis of the anaemia. However, as the marrow cannot respond adequately to the shortened red cell survival because of impaired Epo production and the ability of the red cell progenitors to respond to Epo, it is an aggravating factor contributing to the anaemia.

The various factors that play a role in the pathogenesis of the anaemia of infection are illustrated in Fig. 1.

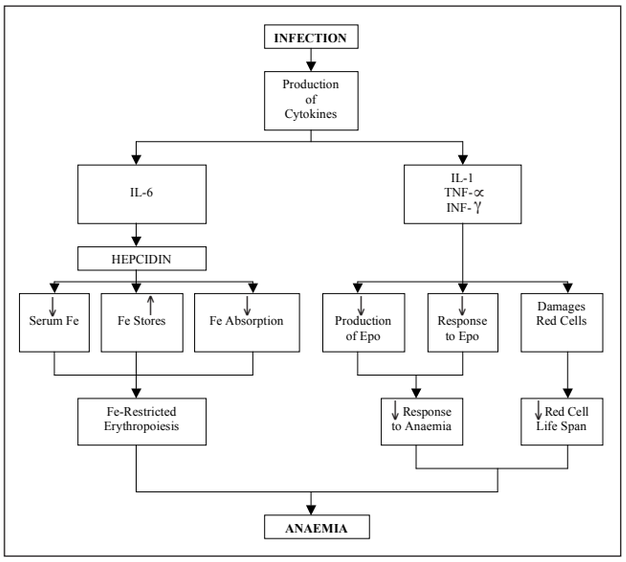

Fig. 1. The pathogenesis of the anaemia of infection (IL-1 = interleukin-1; IL-6 = interleukin-6; TNF-α = tumour necrosis factor-alpha; INF – γ = interferon-gamma; Fe = iron; Epo = erythropoietin).

Diagnosis

Clinically the anaemia may be manifest by pallor, and result in weakness and fatigue. The anaemia is generally mild to moderate, with the haemoglobin concentration 8 - 9 g/dl, and the severity correlates roughly with the activity of the associated infection. The anaemia is usually normocytic, with normal mean cell volume (MCV), and normochromic with normal mean cell haemoglobin (MCH), but in 20 - 50% of patients it is mildly microcytic (reduced MCV) and hypochromic (reduced MCH). Marked hypochromia and microcytosis are unusual and generally indicate an associated abnormality such as iron deficiency or thalassaemia. The reticulocyte count is low for the degree of anaemia, indicating inappropriate production of red cells to compensate for the anaemia.

The diagnosis of AI can usually be confirmed by the changes in the tests of iron status. The serum iron is low, transferrin

concentration normal to low, and transferrin saturation low to normal. Serum ferritin is raised as part of the acute-phase reaction.[12]

Differentiation of the anaemia of infection from iron deficiency anaemia

When the AI is microcytic and/or hypochromic, it is important to distinguish it from iron deficiency anaemia (IDA), as iron therapy is contraindicated in AI but necessary in IDA. In both AI and IDA, the serum iron concentration is decreased. Serum transferrin is usually high in IDA, but low in AI. In IDA the reduced serum iron with raised transferrin results in low percentage transferrin saturation (serum iron divided by transferrin), whereas in AI, both serum iron and transferrin are reduced, so that saturation is either normal, or only slightly reduced. Serum ferritin is typically low in IDA but raised in AI, as part of the acute-phase reaction.

Differentiation between IDA and AI can usually be made on the basis of the above tests, but may at times be difficult. In that situation, some of the newer tests of iron status may be of value in the differentiation of IDA and AI, in particular measurement of serum-soluble transferrin receptors (sTfr) and red cell zinc protoporphyrin (ZPN). sTfr are responsible for the uptake of transferrin-bound iron by cells in the bone marrow, and increase with iron deficiency. Serum sTfr, unlike ferritin, are not usually affected by infection, and are therefore useful in the differentiation of AI from IDA.[13] More recent studies, however, suggest that the differentiation is not always absolute, as sTfr may sometimes increase with infection.[14]

In iron deficiency, with insufficient iron to insert into protoporphyrin to make haem, zinc is incorporated into protoporphyrin instead of iron. Thus ZPN increases in iron deficiency. Measurement of ZPN may be of value in the differentiation of IDA and AI, as the concentration does not increase in infection, but as with sTfr, this is not always the case.

Other tests of value in the differentiation include measurement of C-reactive protein and the ESR, which are usually raised in bacterial infection but normal in uncomplicated iron deficiency.

In iron deficiency, tissue iron stores are depleted, while in infection they are normal or increased due to the diversion of iron from plasma to stores. Determination of iron stores will therefore usually differentiate IDA from AI, and is probably the gold standard for the distinction of the two entities. Iron stores in the reticuloendothelial system (RES) can be assessed directly by staining a bone marrow aspirate for iron. However, bone marrow aspiration is an invasive procedure, and is rarely indicated, and then only when all other tests fail to make the distinction. Despite being semi-quantitative and somewhat subjective, assessment of iron in a bone marrow aspirate remains the best determinant of the presence or absence of iron deficiency. Finally, response to iron supplementation is considered the ultimate diagnostic test for iron deficiency.

Combined anaemia of infection and iron deficiency

The detection of concomitant iron deficiency in patients with AI, a combination that is not uncommon, is usually difficult. The specificity for iron deficiency of the transferrin concentration and percentage saturation is poor when there is associated infection. Low serum ferritin, the hallmark of iron deficiency, is also of limited value in the detection of iron deficiency in infection, because of the rise in ferritin

Table I. Haematological and biochemical changes in anaemia of infection, iron deficiency anaemia and combined infection and iron deficiency

	Anaemia of infection	Iron deficiency anaemia	Combined infection/ iron deficiency
Haematology			
Haemoglobin	↓	↓	↓
MCV	N or ↓	↓	↓
MCH	N or ↓	↓	↓
RCC	↓	↓ or N	↓
Marrow iron	N or ↑	↓	↓
ESR	↑	N	↑
Biochemistry			
Iron	↓	↓	↓
Transferrin	↓ or N	↑	↓
Transferrin saturation	↓	↓	↓
Ferritin	N or ↑	↓	N or ↓
Soluble transferrin receptor	N	↑	N or ↑
Zinc protoporphyrin	N or ↑	↑	N or ↑
CRP	↑	N	↑

MCV = mean cell volume; MCH = mean cell haemoglobin; RCC = red cell count; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; N = normal.

in infection. Thus in IDA with infection, serum ferritin concentration may be towards the lower end of the reference range, but not frankly abnormal. Patients with ferritin concentrations as high as >50 µg/l may be iron deficient, although those with concentrations >100 µg/l are usually iron replete.

The changes in these various tests in AI, IDA and combined AI and IDA are summarised in Table I. Whereas the identification of concomitant iron deficiency in some of the anaemias of inflammation, e.g. that which occurs in rheumatoid arthritis has significant clinical implications due to the value of therapeutic iron, it is probably of much less significance in the anaemia of bacterial infection, in which the use of therapeutic iron is at best controversial, and at worst detrimental by exacerbating the infection. It is for this reason also that a therapeutic trial of iron, the ultimate diagnostic test for iron deficiency, is not feasible when there is infection.

Treatment

Treatment of the AI is primarily the treatment of the underlying infection with appropriate anti-infective therapy. Treatments aimed specifically at correcting the anaemia have varied. Where a cure of the underlying infection is impossible, transfusions for rapid correction of haemoglobin levels and human recombinant Epo (rhEpo) can be used with varying success.

Red cell transfusion in the presence of ongoing infection is usually only a temporary expedient that elevates the haemoglobin concentration in the short term, but does not address the underlying disorder. Red cell transfusion is indicated in life-threatening anaemia (Hb <6.5 g/dl) or when anaemia is particularly severe (Hb <8.0 g/dl). In general, red cell transfusions should be used to maintain the haemoglobin concentration at 7.0 g/dl or greater.

The role of therapy with rhEpo in AI is largely untried and is not recommended as a specific treatment for the AI. There is no detailed literature on its use specifically in infections other than HIV. In one report,[3] the administration of rhEpo to patients with osteomyelitis receiving parenteral antibiotics significantly decreased the need for red cell transfusions.

Treatment with iron is contraindicated in uncomplicated AI. The use of iron when IDA is present in addition to AI is controversial, owing to the promotion of bacterial growth by iron. In general, iron therapy should only be considered if there is severe concomitant iron deficiency. Treatment should be with oral iron only and never with parenteral iron due to the possible aggravation of infection by a large bolus of iron.

Vitamin B_{12} and folic acid have no place in the treatment of AI, unless there is clear evidence of concomitant deficiency. Folic acid should not be given to patients receiving antibiotic treatment with sulphasuxazole-trimethoprim. This antibiotic exerts its antibacterial effect by inhibition of folate synthesis in bacteria, an action that may be bypassed by the provision of pharmacological doses of folic acid.

The recognition of the pivotal role of hepcidin in iron metabolism and in the pathogenesis of AI could lead to the development of inhibitors of hepcidin for clinical use. Such hepcidin antagonists could be expected to release the iron sequestered in stores, restore the supply of iron to the marrow and improve the anaemia. However, the release of iron could aggravate the infection, thus triggering a therapeutic dilemma. Of interest also is the report that alcohol reduces levels of IL-6, and thus would be expected to reduce the hepcidin effect and possibly reduce the degree of anaemia.[15]

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