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Interaction of Inflammatory Cytokines and Erythropoeitin in Iron Metabolism and Erythropoiesis in Anaemia of Chronic Disease

M. JONGEN-LAVRENCIC, H.R.M. PEETERS, G. VREUGDENHIL , A.J.G. SWAAK

Summary **In chronic inflammatory conditions increased endogenous release of specific cytokines (TNFα, IL-1, IL-6, IFNγ and others) is presumed. It has been shown that those of monocyte lineage play a key role in cytokine expression and synthesis. This may be associated with changes in iron metabolism and impaired erythropoiesis and may lead to development of anaemia in patients with rheumatoid arthritis.**

Firstly, increased synthesis of acute phase proteins, like ferritin, during chronic inflammation is proposed as the way by which the toxic effect of iron and thereby the synthesis of free oxy-radicals causing the damage on the affected joints, may be reduced. This is associated with a shift of iron towards the mononuclear phagocyte system which may participate in the development of anaemia of chronic disease.

Secondly, an inhibitory action of inflammatory cytokines (TNFα,IL-1,) on proliferation and differentiation of erythroid progenitors as well as on synthesis of erythropoietin has been shown, thereby also contributing to anaemia.

Finally, chronic inflammation causes multiple, complex disturbances in the delicate physiologic equilibrium of interaction between cytokines and cells (erythroid progenitors, cells of mononuclear phagocyte system and erythropoietin producing cells) leading to development of anaemia of chronic disease (Fig.1).

Key words **Anaemia of Chronic Disease, Erythropoietin, Cytokines, Iron Metabolism**

INTRODUCTION

Anaemia of chronic disease (ACD) is often observed in patients with chronic inflammatory, malignant and infectious disorders (1,2). It has been studied most extensively in rheumatoid arthritis (RA) (3-6).

One of potential mechanisms involved in development of ACD is the shift of iron towards the storage compartment and impaired iron transport to the erythroblast (7-9). Iron is an essential element that participates in haemoglobin synthesis. Iron balance is achieved in the body by regulation of iron absorption and recycling of the majority of total body iron stores (10). It is considered that in inflammatory conditions changes in iron metabolism occur which may partly be mediated by cytokines (11). Tumour necrosis factor-alpha (INFα), interleukin 6 (IL-6), IL-1 and interferon gamma (IFNγ). TNFα, IL-1 and IFNγ (12-14) are supposed to play an important role (Table I).

Control of bone marrow cell production involves complex interactions between haematopoietic cells, accessory cells in the bone marrow micro-environment, and an interaction of cytokines that either promote or suppress cell proliferation (15). The fine regulation of erythropoiesis is effected by erythropoietin (Epo). Epo is produced mainly by the kidneys (16) but also extrarenally by macrophages (17,18). Proliferation and haemoglobinization of erythroid progenitors is inducible by Epo in vitro (19). Inappropriately low serum Epo levels have been reported in anaemic RA patients compared to those found in patients with uncomplicated iron deficiency anaemia of equal severity (20-23). This relative Epo deficiency supports the concept of impaired Epo response

Department of Rheumatology, Dr. Daniel den Hoed Clinic, The Netherlands
*Department of Internal Medicine, St. Joseph Hospital Eindhoven, The Netherlands

Table I: *Effects of cytokines and erythropoietin on iron metabolism and erythropoiesis in ACD reported in the literature*

	TNFα	references	IL-1	references	IFNγ	references	Epo	references
Iron absorption	↓	29	?		?		↑	40,41
Ferritin synthesis/function								
- gene activation	↑	32,43,44	↑	13,43,44,92	↓	93	?	
- function of iron transport (reduction of Fe^{3+} to Fe^{2+})	?		?		↑	11,33,39	?	
Transferrin								
- synthesis	?	94	?	94	?	94	↑	24,25,26,40
- microheterogeneity	↑	55,56	↑	55,56	?		?	
- cell-receptor expression	?	49	?		↑	93	↑	26,40
- serum-receptor level	?	50	?		?		↑	26
Erythroid progenitors								
- BFU-e, CFU-e growth	↓	30,55,72,74	↓	77,80	↓	77,81,82	↑	84,86,90
Erythropoietin								
- synthesis	↓	85,87	↓	85,87	?		↓	86
- receptor expression	?	91	?		?		↓	86

up-regulation/ stimulation (↑), down-regulation/suppression (↓), uncertain/ not-studied (?)

to anaemia and justifies the recent attempts to treat ACD with recombinant human Epo (rhEpo) (24-26). Furthermore, some potent inhibitors of erythropoiesis have been described. Several cytokines like TNFα, IL-1, IL-6, IL-8, IFN_β and IFNγ which mediate chronic inflammation in rheumatoid arthritis (RA)(27) and chronic infections appear also to contribute to disturbed erythropoiesis and ACD (28). This paper reviews new aspects on the role of cytokines and Epo in iron metabolism and erythropoiesis with respect to the development of ACD (Table I, Fig. 1).

Iron metabolism

Absorption and reutilisation

A number of changes in iron metabolism occur during the inflammatory response. These changes include decreased absorption of iron by the intestinal mucosa (29), increased synthesis and content of ferritin within cells of the mononuclear phagocyte system (MPS) (30), increased transferrin receptor mRNA and transferrin receptor protein synthesis (31) and a block of iron release from macrophages (7).

Inflammation is characterized by increased production of TNFα and current evidence indicates that this cytokine is a major modulator of changes in iron metabolism as observed during inflammation (32). The way by which TNFα affects macrophage iron handling remains to be elucidated. It may be argued that TNFα causes increased degradation of intracellular ferritin, leading to the formation of haemosiderin, from which iron would be less easily liberated for subsequent extracellular release (12). Torti et al. (11) showed that TNFα induces activation of the ferritin heavy chain gene. Higher amount of L-ferritin with high affinity for Fe may explain reduced availability of iron released from the MPS (11). In addition, Roger et al. (13) have reported that IL-1 also increases ferritin production with the same iron-retaining effects. Finally, one should consider that the main source of TNFα and IL-1 is the macrophage itself, thus suggesting that the effect of TNFα and IL-1 on macrophage iron metabolism is explained by an autocrine mechanism. TNFα might in fact act as a normal physiological regulator of macrophage iron metabolism, and abnormal iron retention occurs only when excessive amounts of TNF& are produced during inflammatory processes.

During recent years, data have been generated suggesting that also IFNγ inhibits erythropoiesis in vitro, probably by influencing iron metabolism (33). Enhanced concentrations of IFNγ were found in patients with chronic inflammatory disorders (34,35). Levels of neopterin, which serves as a marker for activation of macrophages by IFNγ, are increased in a variety of infectious, inflammatory and malignant disorders (36-38). It was proposed that dihydroneopterin catalyses the reduction of Fe^{3+} to Fe^{2+} which is required for transfer of iron from transferrin to apoferritin (11). In that way neopterin derivates may be involved in the recruitment of iron from the circulation. In addition, neopterin may support the stabilization of ferritin mRNA (39). Thus, pteridine derivates such as neopterin may at least play an indirect role in controlling iron metabolism, and in circumstances of chronic inflammation pteridin may facilitate the transfer of iron into activated monocytes/macrophages. This may have an antiproliferative action upon erythropoiesis.

Epo is supposed to be involved in the dynamics of iron metabolism as well. The amount of body iron stores is

the major factor controlling the absorption of iron from the gastrointestinal tract. Furthermore, the degree of erythropoiesis is also believed to influence iron absorption but the mechanism is unknown. It was demonstrated that administration of rhEpo induces a decline in the labile iron compartment and an increase in reticulocyte count, haematocrit and serum transferrin receptor (40). Both factors could have produced enhanced absorption of iron (41).

Cytokines and iron transport

Ferritin

Increased ferritin synthesis is a primary nonspecific response which is part of a general pattern of the systemic effects of inflammation (30). The expression of acute-phase protein genes (including ferritin) in the liver is controlled by the action of cytokines (42). Prominent stimulatory effects have been ascribed to IL-1, IL-6 and TNFα (43).These data suggest that TNF and IL-1 affect a subset of acute phase plasma protein genes, including ferritin, via cytokine-specific signal pathways (44). Indeed ACD is characterised by a marked increase in serum ferritin level up to 250% of normal values (6).
Isotypes of ferritin (H or L) are not definitely established. There are some reports (Immune-alkaline phosphatase staining of bone marrow cells using monoclonal antibody specific for the H and L subunit of ferritin) suggesting that the erythroblasts of patients with ACD contain higher amounts of ferritin, present in both H and L forms and that MPS cells of those patients have higher contents of L-ferritin type, compared to normal subjects (45).
Higher amounts of L-ferritin with high affinity for iron could explain the reduced iron release from MPS. Furthermore, accumulation of iron in erythroblast, in form of H-ferritin, may participate in inhibition of proliferation of erythroblast and lead to the anaemia (45).
The presence of extracellular ferritin, particulary the H type, inhibits the proliferation of haematopoietic progenitor cells in vitro (46). Therefore it is not unlikely that in chronic inflammation release of this type of ferritin may contribute to development of ACD.

Transferrin

Transferrin is a negative acute phase protein (47) and in patients with active RA and ACD its levels are decreased (3,4,6). Transferrin plays a key role in the process by which cells acquire iron for growth and haemoglobin synthesis. Transferrin iron saturation, the affinity of transferrin for the receptor and the number of transferrin receptors expressed by erythroblast determine erythroblast iron uptake.
It has been shown that iron uptake and transferrin binding by erythroblasts (48) as well as transferrin receptor expression (49) and serum transferrin receptor level (50-52) are reduced in patients with active RA accompanied by ACD. It has been shown that some acute phase proteins like α1-anti-trypsin and α2-macroglobulin, which are increased in chronic inflammation, were able to diminish affinity of transferrin receptor for transferrin and to suppress internalization of iron binding to transferrin (53,54).
Regulation of synthesis and glycosylation of transferrin and other acute phase proteins is proposed to be mediated by TNFα, IL-1, IL-6 and transforming growth factor β (55,56), but the mechanism has not yet been fully elucidated. Current data suggest that transferrin exists in different microheterogenetic forms (57). The functional properties of transferrin, such as affinity to its receptor can be modulated by this phenomenon. Altered biological activity of transferrin may possibly influence the capability of iron delivery to the erythroblasts. Structural variation in transferrin glycosylation has been considered in chronic disease such as RA (58). A significant shift in the microheterogenety pattern of transferrin was suggested in ACD, reflecting increased synthesis of transferrin with highly branched glycan chain (58), associated with a higher affinity to its receptor. The increased synthesis of highly sialyated transferrin, in view of the impaired erythroblast iron availability and total transferrin synthesis in ACD, may therefore be seen as a part of a compensatory mechanism aiming at facilitating iron transport to erythroblasts.
In RA the existence of ACD and alteration in the glycosylation pattern of transferrin appears to correlate with disease activity. One might therefore postulate that cytokines such as IL-6, TNFα and IL-1 play an important role in changing iron metabolism not only by inducing ferritin synthesis, but also by modifying transferrin glycosylation and transferin receptor numbers.

Lactoferrin

Lactoferrin is found in neutrophil granules (59). Higher serum concentrations may be present during inflammation as a result of neutrophil degranulation (60). The role of lactoferrin in the development of ACD is not yet clear. Lactoferrin is involved in modulating a number of immune responses, as it inhibits granulopoiesis (61), suppresses antibody production (62) and natural killer cell activity (63). Furthermore, as shown recently, lactoferrin prevents recruitment and activation of leucocytes in

sites of inflammation and inhibits the production of both IL-1 and TNF by a negative feedback mechanism (64). Lactoferrin therefore may act as a protective factor against exacerbation of RA and its complications (ACD).
However, lactoferrin may participate in a minor degree in the development of ACD. Iron-lactoferrin could not substitute for iron-transferrin (65) and iron trapping by lactoferrin may result in decreased iron availability for erythropoiesis.

Erythropoiesis in ACD

Erythroid progenitors

There is recent evidence of impaired synthesis of haemoglobin in erythroblasts of patients with ACD (66). Since, 5-aminolaevulinate (ALA) synthase is an important enzyme in the biosynthetic pathway of haem, Houston et al. studied the synthesis of 5-aminolaevulinate (ALA) synthase activities in erythroblasts of patients with RA and anaemia, and showed significantly reduced activity of ALA-synthase and increased protoporphyrin levels in erythroblasts of patients with ACD (66). The therapy of ACD with r-h-Epo was shown to be associated with increased activation of ALA-synthase, synthesis of haem and correction of anaemia (67).
A number of investigators reported a decreased growth of burst forming units-erythroid (BFU-e) and colony forming units erythroid (CFU-e) in vitro in patients with ACD compared to normal controls (4,23,68) whereas others did not demonstrate significant differences (69,70).
RA is associated with continuous macrophage activation. Although resting macrophages, in physiologic numbers, enhance erythroid colony formation in vitro, increased numbers of activated macrophages markedly inhibit CFU-e and BFU-e growth (71). As mentioned above, activated macrophages produce a number of cytokines which may affect erythropoiesis, including IL-1, IFNα,β,γ and TNFα (27). Of these, TNFα is a potential candidate as a macrophage mediator of ACD. In vitro, inhibitory effects of TNFα on human CFU-e and BFU-e have already been proven (30,72-74). Whether these effects of TNFα on erythroid progenitors are direct (29) or whether the presence of other factors and cells is required (75,76) is not entirely clear. In addition, chronic TNFα exposure was shown to suppress erythropoiesis in vivo (32,77). Nude mice inoculated with Chinese hamster ovary (CHO) cells expressing the human TNFα gene developed a hypoferremic, hypo-proliferative anaemia with normal iron stores and decreased numbers of bone marrow and splenic CFU-e and BFU-e (32). TNFα concentration was shown to be elevated in RA patients with ACD and correlated well with RA disease activity (78). It may be argued that TNFα plays a specific role in ACD, based on the inverse correlation of serum TNFα and haemoglobin in the above mentioned study (78) as well as in a group of HIV patients (79).
IL-1 has also been shown to inhibit erythropoiesis in vitro and in vivo and has been implicated in ACD (77,80). Maury et al. (79) have reported that IL-1 levels are significantly elevated in anaemic RA patients as compared with RA patients without anaemia. Means et al. (77) investigated the inhibition of human CFU-e colony formation by IL-1 and showed that inhibition of unpurified marrow CFU-e was indirect and was mediated by IFNγ.
We have already discussed the function of IFNγ in iron metabolism. The effects of IFNγ on erythroid progenitors have also been studied. Mamus et al (81), looking to BFU-e and CFU-e colony growth, reported that the inhibitory effect of IFN-γ was indirect and required accessory cells, while Raefsky et al. (82) and Means et al. (77) reported that this was a result of direct action of IFNγ on CFU-e.

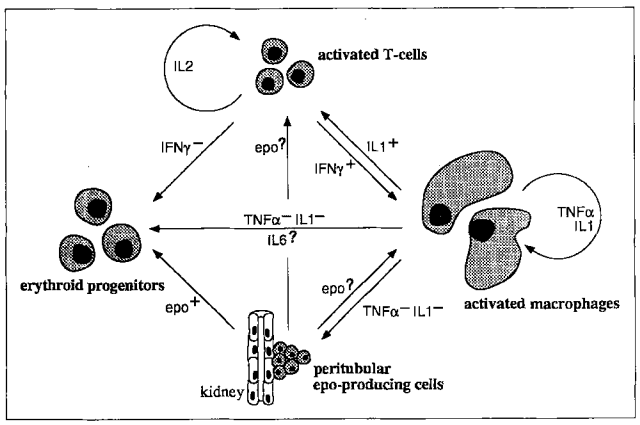

Fig. 1: Role of cytokines and erythroietin on the erythropoiesis.

Erythropoietin

Recent evidences suggest that the serum Epo level is low in relation to the haemoglobin concentration in anaemic patients with acute or chronic infection (20,22,83) and inflammation or tumours (84,85), thus showing a blunted response to the anaemia.
Impaired synthesis of Epo, in response to hypoxic stimulus, may contribute to persistence of anaemia. Late in the course of erythroid progenitor cell differentiation, the cell enters a period in which it depends upon Epo to prevent apoptosis (17). The degree of apoptosis may be determined by several factors, including the circulating erythropoietin concentration, the relative number and

the affinity of the Epo-receptors to its ligand (86). In response to anaemia; Epo production by the kidney increases. This leads to raised serum Epo levels being experienced by those progenitor cells entering the Epo-dependent period which is sufficient to prevent their apoptosis. This results in survival and differentiation of erythroid progenitors into erythrocytes. That may argue for the hypothesis that increased concentration of Epo in ACD is not high enough to drive a sufficient number of erythroid progenitors to cell proliferation, thus resulting in underproduction of erythrocytes and persistence of anaemia.

It was proposed that cytokines play a role in the pathogenesis of Epo deficiency in mentioned disorders. Jelkman et al. (85) showed that IL-1β, IL-1α and TNFα decreased Epo production of human hepatoma cell line HepG2 and Hep3B (87) in hypoxic condition and that IL-1β can block Epo formation in isolated serum-free perfused rat kidney (85).

ACD can be corrected with rhEpo therapy in patients with RA (24-26,48,88). In mice, it was already demonstrated that anaemia, caused exclusively by chronic TNF-exposure, could be corrected by administration of exogenous Epo (89). In vitro experiments on bone marrow of RA patients suggested that suppressive effects of TNFα on BFU-e and CFU-e growth could be partly corrected by the addition of excess rhEpo to the cultures (90). Therefore one may conclude that the beneficial effects of Epo on ACD in RA patients can be explained, at least to some extent, by the ability of Epo to counteract cytokine-mediated suppression of erythropoiesis.

Advances in knowledge regarding Epo-receptor structure and function are beginning to provide better understanding of human diseases that affect erythropoiesis but there is no direct evidence of signal transduction pathway disturbances of Epo-receptor in ACD.

Modulation of expression and function of Epo receptor by cytokines, like TNFα (91), IFNγ or IL-1, may be one possible explanation of strong decrease of BFU-e in vitro if TNFα is added in the culture system. Whether this is a direct effect of TNF (downregulation of Epo-receptor numbers on erythroblasts) or an indirect process induced by production and activation of other cytokines, remains to be established.

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Correspondence to: A. SWAAK,
Dept. of Rheumatology,
Dr.Daniel den Hoed Clinic,
Postbus 5201, 3008 AE Rotterdam,
THE NETHERLANDS