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ANEMIA OF CHRONIC DISORDERS IN SYSTEMIC AUTOIMMUNE DISEASES

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ABSTRACT

Background and Objective. Anemia of chronic disorders (ACD) is a mild to moderate anemia characterized by decreased serum iron, decreased total iron-binding capacity and increased iron stores that occurs in a wide variety of diseases including cancer, chronic infections and inflammatory disorders. The reason that prompted us to write this review is two-fold. First, systemic autoimmune diseases are frequently characterized by ACD. Second, advances in pathophysiology of systemic autoimmune diseases as well as pathogenesis and treatment of ACD have remained separate. Consequently, the approach to the evaluation of ACD in systemic autoimmune disorders is usually either immunology- or hematology-oriented. The aim of this review is to consolidate the pertinent information from both fields to obtain a more complete definition of ACD.

Information sources. The articles reviewed have been published in journals listed in the Science Citation Index© and Medline©. In addition, the authors have a vast experience in hematology systemic autoimmune disorders.

State of art and Perspectives. ACD is a parameter of disease activity in systemic autoimmune diseases. The severe inflammatory stimuli responsible for the pathophysiology of these disorders lead to several systemic changes (referred to as chronic active phase response) aimed at coping with chronic tissue injuries. These reactions are brought about by inflammation-associated cytokines, like IL-6, IL-1, TNFα, TGFβ regulating the liver synthesis of acute phase proteins. Many cytokines involved in chronic acute phase response, including IL-1, TNFα, TGFβ, inhibit *in vitro* erythroid colony formation. In addition, circulating TNFα is elevated in rheumatoid arthritis (RA), IL-1β serum levels are significantly increased in RA with ACD and RA patients treated *in vivo* with antibodies (Abs) to TNFα improve, with an increase in Hb values. Reduced erythropoietin (EPO) activity usually due to reduced production is relevant in the pathogenesis of ACD observed in systemic autoimmune diseases. Both the production and the action of EPO may fall under the control of IL-1 and IFN-γ. The most controversial and stimulating aspect in the pathogenesis of ACD in systemic autoimmune disorders is the role of iron metabolism and nitric oxide (NO) that contributes to the regulation of iron cellular metabolism. Both iron deficiency and iron overload may influence the proliferation of B and T lymphocytes and differentially affect T helper (TH)-1 and TH-2 lymphocytes. Further, TH-1 cytokines stimulate and TH-2-type cytokines inhibit NO production. Based on these premesis, cell-mediated immunity may be expected to have influence on NO synthesis and the mechanisms leading to iron accumulation in the reticulo-endothelial system.

Key words: anemia, chronic disorders, autoimmunity, cytokines, erythropoietin, soluble transferrin receptor

Anemia of chronic disorders (ACD) is a mild to moderate normocytic-normochromic or, less frequently, microcytic-hypochromic anemia characterized by decreased serum iron, decreased total iron-binding capacity and increased iron stores.[1] Reticulocytes levels are normal to reduced and classify ACD among hyporegenerative anemias.[1] Consequently, one might ask which mechanisms cause the impaired erythropoietic response in ACD. Altough ACD is a frequent and easily diagnosed clinical entity, its pathogenesis still remain unclear. Impaired iron release from the reticuloendothelial storage, reduced supply of erythropoietin (EPO) to the bone marrow (BM) and inadequacy or defects of BM erythroid precursors have been postulated to be the major pathogenetic processes,[1] while slightly reduced red cell survival has proved to be minimally influent.[2] Whatever pathogenetic mechanism is operating in ACD, the final result is a diminished erythropoiesis as also sustained by the low levels of soluble transferrin receptor (sTfR).[3,4] Measurement of sTfR, mainly derived from immature erythroblasts, is a simple, non-invasive method to investigate the rate of erythron activity that strongly correlates with ferrokinetic measurements of erythropoiesis: in the absence of iron deficiency, it is the best estimate of total erythropoiesis.[5-7]

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ACD owes its name to the fact that it accompanies a very broad spectrum of diseases most commonly represented by cancer, chronic infections and inflammatory disorders, but that may even include congestive heart failure.[8] The high incidence and the heterogeneity of *chronic disorders* that may present ACD have two implications. First, ACD is one of the most common forms of anemia. Second, differences are to be expected in the pathogenesis of and potential therapeutic approaches to ACD occurring in different clinical settings. Systemic autoimmune diseases are frequently characterized by ACD, and may benefit from a more unified immunological and hematological definition. Therefore, it can be useful to review a number of informations that relate erythropoiesis to immune hyperactivity. More specifically, there are three areas of major interest: a) the organism's reaction to inflammatory stimuli and the effect of proinflammatory cytokines on erythropoiesis; b) the production and activity of erythropoietin (EPO); c) the possibility that cell-mediated immunity may influence iron metabolism through its effects on NO synthesis.

ACD and acute phase response

Inflammatory stimuli are the basic pathophysiological elements of systemic autoimmunity. A useful starting point is therefore to analyze how the organism reacts to these stimuli and how these reactions may be related to the development of ACD. Mild inflammatory stimuli promote a local response. Severe inflammatory stimuli cause a number of systemic changes referred to as acute phase response which may be transient or persist and evolve into the so-called *chronic acute phase response*. This unfortunate (but commonly used) name defines a host of reactions brought about by several cytokines through which the organism tries to cope with chronic or relapsing tissue injuries (reviewed in ref. #9). Inflammation-associated cytokines regulate liver synthesis of acute phase proteins, in which a prominent role is played by the pentraxin C reactive protein (CRP).[9] IL-6 is a mediator of major importance[10] together with other members of the family of distantly related cytokines that use gp130 as common signal transducer pathway, like oncostatin M (OM), leukemia inhibiting factor (LIF), ciliary neurotrofic factor (CNTF) and IL-11.[10] IL-1, TNFα and TGFα[1,11] also play a major role. The identification of new members of the pentraxin gene family, like ptx3, whose expression is not controlled by IL-6 and occurs in organs besides the liver,[12] may shed further light onto the complexity of the system. The final result of these reactions, including fever, changes in plasma protein concentration, altered synthesis of proteins and hormones, is to reset several homeostatic settings. Though the adaptive purpose of these reactions is far from established, they are usually presumed to improve the possibilities of defense and repair. The high levels of CRP favor the identification, binding and clearance of pathogens and phospholipids released by damaged cells.[13,14] The synthesis of ferritin is increased by IL-1, TNFα and IL-6;[9] the synthesis of hepatic transferrin is downregulated. Both events result in low levels of circulating iron that are deemed useful in infectious processes because they withhold iron from invading microorganisms.[15] Along the same vein, transferrin may also be synthesyzed by activated macrophages and has been shown to stimulate the local proliferation of lymphocytes thereby avoiding the potentially detrimental effects of hypoferremia on the immune system.[16]

The complexity of cytokine networks and their redundance do not allow to precisely pinpoint the exact role individually played by each cytokine in the different aspects of chronic acute phase response. Cytokines are rapidly cleared, locally translated and antagonized by soluble receptors and anti-cytokine antibodies (Ab): a consequence of practical importance is that a limited significance can be ascribed to the measurement of cytokines in the serum.[17]

Notwithstanding these difficulties, a number of cytokine-mediated features of the chronic acute phase response in systemic autoimmune diseases may be taken to explain some hematological abnormalities. In several autoimmune diseases, that include polymyalgia rheumatica, Still's disease and Behcet's disease, both leukocytosis and thrombocytosis are frequently present and it is not unreasonable to ascribe these modifications to the activity of IL-8 and/or of G-CSF.[18] It is pertinent to the present discussion the observation that ACD frequently accompanies systemic lupus erythematosus (SLE) flare up and represents a useful parameter of disease activity.[19]

Interestingly, in the vast majority of exacerbations of SLE (with the notable exceptions of serositis and arthritis) CRP is not increased.[14] CRP levels in SLE help in discriminating the immune inflammation due to the exacerbation of the disease from the inflammation triggered by infections or thrombotic events where CRP is increased.[20] Though the molecular basis for the lack of increase of CRP in SLE immune inflammation is presently unknown, it has been ascribed to lower levels of TNFα and/or IL-6, to the interference of soluble receptors or Abs with TNFα and/or IL-6 or to the reduced production of other cytokines like IL-11.[17,21]

Cytokines and inhibition of erythropoiesis in systemic autoimmune diseases

The majority of *in vitro* data on the potential role of cytokines in the pathogenesis of ACD in chronic

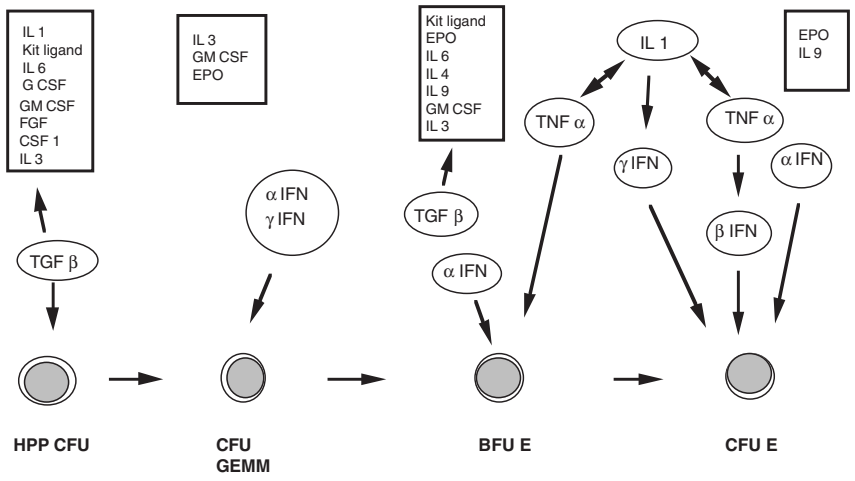


Figure 1. Cytokines that influence erythropoiesis with a stimulatory (rectangular frame) and an inhibitory (oval frame) activity.

inflammatory disorders have been obtained in rheumatoid arthritis (RA). In the early eighties it was shown that RA erythroid colonies are essentially normal and that the serum, but not adherent cells, from anemic RA patients can inhibit erythroid colony formation *in vitro*.[22] Understandably, the addition of whole serum, instead of purified cytokines, led to a significant variability of results.

Many cytokines involved in chronic acute phase response have an inhibitory activity on erythroid colony formation *in vitro*. The cytokines that have a major inhibitory effect on red cell precursors in the bone marrow (BM) are shown in Figure 1. TGFβ inhibits the earliest erythroid precursors and BFU-E both directly and indirectly by inhibiting the activity of IL3.[23,24] The inhibitory effect of TNFα on the growth of BFU-E and CFU-E is indirect, at least partly mediated by the IFNβ produced by BM stromal cells.[25] Likewise indirect is the inhibitory effect of IL-1 on CFU-E generation and is mediated by the IFNγ produced by T cells.[26] The cytokines that negatively regulate erythroid precursors act synergistically, as consistently reported for IL-1 and TNFα.[11]

The role of IL-1 on erythropoiesis is contradictory.[11] The chronic administration of IL-1 inhibits the more differentiated precursors (BFU-E and CFU-E);[27] on the contrary, a single administration of IL-1 stimulates the more immature precursors (HPP-CFU).[28,29] The positive effect of low doses of IL-1 appears to be mediated by G-CSF, GM-CSF and IL-3 that is also able to contrast the inhibitory effect exerted by IFNγ on BFU-E.[30,31] Experiments in mice have documented a protective effect of IL-1 against the chemo-and radiotherapy-induced BM toxicity.[32,33] Further, IL-1 and kit ligand synergize to increase DNA synthesis in erythroid precursors.[34]

The majority of data on the potential role of cytokines in the pathogenesis of ACD in systemic autoimmune disorders have been obtained with experimental studies *in vitro* that have been extrapolated to explain the pathogenesis of *in vivo* response. Circulating TNFα is elevated in RA[35] and IL-1β serum levels are significantly increased in RA with ACD as compared to RA without anemia,[36] suggesting the possibility that they might contribute to the reduced erythropoietic activity. RA patients treated *in vivo* with monoclonal Abs to TNFα respond with a clinically apparent improvement of the disease that includes an increase in Hb values.[37,38] IL-1β serum levels in SLE are of marginal significance, while a high serum concentration of IL-1 receptor antagonist (IL-1Ra) appears to be a good indicator of disease activity.[39] It still has to be clarified whether high levels of IL-1Ra may represent a compensatory mechanism that protects against an excess of IL-1 and how the interplay of T helper (TH)2-type cytokines may influence the IL-1/IL-1Ra balance.[40] Another unresolved question is whether the proinflammatory cytokines that negatively regulate erythropoiesis flush in the BM from outside or whether they are produced and released within the BM and locally exert their effect. The latter possibility is in keeping with the observation that SLE BM macrophages have an abnormal pattern of cytokine production as compared to macrophages obtained from normal BM.[41]

In vitro, IL-6 has been shown to have a positive effect on erythropoiesis.[42] However, this experimental finding is in marked contrast with the clinical observation that high levels of IL-6 are detectable in the serum of patients with active RA and, even more specifically, in patients with RA and ACD.[42,43] Taking into account the results of clinical trials with recombinant human IL-6 in patients with malig-

nancies,[44] it has been hypothesized that the hyperproduction of IL-6 might be responsible of ACD in patients with systemic onset juvenile chronic arthritis (SOJCA), through a reduced availability of iron to erythroid precursors.[45] Actually, IL-6 levels are significantly increased also in a number of other autoimmune disorders that include RA and SLE,[46-48] where ferritin levels are (modestly) increased, sTfR values are low, EPO levels are inadequate and microcytosis is absent. On these grounds, it is safe to consider IL-6 as a useful inflammatory marker of disease activity, that is not directly responsible for the development of ACD.

EPO and ACD in systemic autoimmune diseases

EPO favors the entry of BFU-E into the cell cycle and prevents CFU-E from undergoing apoptosis;[49] the most EPO-sensitive cell, i.e. the cell with the highest number of EPO receptors, is an erythroid element intermediate between CFU-E and proerythroblast.[50] EPO signalling leads to an increase in the transcription of globin chain genes, the expression of TfR on the red cell surface and the synthesis of membrane proteins.[49] Both the production and the action of EPO may fall under the control of a number of cytokines produced in chronic acute phase response.[50]

A reduced stimulus by EPO appears to play a role in the pathogenesis of ACD observed in systemic autoimmune diseases, even if its relevance may vary in different disorders. Theoretically, the reduced EPO stimulus may depend upon: a) a reduced production of EPO; b) a resistance to EPO-induced stimulus. EPO levels are higher in RA patients with ACD than in non-anemic RA, but the increase in EPO is inappropriate to the degree of anemia.[51] Likewise, we observed a marked EPO deficiency in a series of SLE patients with ACD (unpublished results). In SLE, as well as in a number of other autoimmune diseases, two scenarios may account for the reduced production of EPO, one related to the reversible changes that are linked with the current activity of the disease, the other related to the irreversible changes consequent to cumulative organ damage.[52] At least two possibilities may be envisaged that favor a reduced production of EPO in systemic autoimmune diseases. The first is a cytokine-mediated damage of the interstitial-peritubular area in the kidneys, where EPO is chiefly produced.[53] This possibility is supported by a number of experimental data. Immunohistological studies demonstrate that the kidney interstitial area in patients with SLE and nephropathy is heavily infiltrated by activated $CD4^+$ T lymphocytes and macrophages[54,55] that presumably are cytokine-producing elements. *In vitro*, IL-1 and TNFα have been shown to inhibit at mRNA level the production of EPO.[50,56] EPO mRNA is also inhibited by neopterin,[57] a pteridin increased in the presence of IFNγ-mediated activation of macrophages.[58,59] Another, not mutually exclusive, possibile cause of reduced EPO production is vascular lesion in the kidney peritubular cell area. Such a possibility is suggested by the observation that a vascular interstitial damage is observed in patients with HbS.[60] HbS EPO levels are low for the degree of anemia and, interestingly, decrease with age,[60] thereby indicating that they cannot be uniquely explained by the low oxygen affinity of HbS, but may also be contributed to by the progressive kidney vascular damage. As a thrombotic microangiopathy may occur in the kidneys of patients with antiphospholipid syndrome (APS) and systemic sclerosis (SSc),[61] it is not unreasonable to postulate that, in these instances, the kidney microangiopathy might reduce the ability to produce EPO.

EPO resistance has been shown in a number of *in vitro* and *in vivo* models.[62] A competition for EPO receptors between EPO and proinflammatory cytokines (IFNγ and IL-1) might well be at the basis of EPO resistance. *In vivo*, high levels of proinflammatory cytokines have been detected in patients who do not respond to EPO treatment.[47,63-65] *In vitro*, sovramaximal doses of EPO can correct the IFNγ-induced inhibition of the colony growth of erythroid precursors.[58] These data, taken together, seem to indicate that *in vivo* resistance to EPO might be overcome by increasing EPO dosage. The recent identification of anti-EPO autoAbs in a patient with pure red cell aplasia[66] suggests that another possibile mechanism of EPO resistance might conceivably occur in systemic autoimmune diseases that are characterized by the production of a wide array of autoAbs.

Iron metabolism and nitric oxide in ACD of systemic autoimmune diseases

The significance of the abnormalities of iron metabolism in ACD is controversial. The possibility that, in systemic autoimmune disorders, the hyperactivity of the immune system may, by itself, reduce iron availability to erythroid precursors is attractive, if one considers the relationships between iron abnormalities and the immune system.[57] Both iron deficiency and iron overload may influence the proliferation of B and T lymphocytes and differentially affect TH1 and TH2 lymphocytes.[67] Further, iron accumulation within macrophages appears to increase their cytotoxic effector functions and is believed to provide an advantage in the defense mechanisms against infectious agents and malignancies.[57] It has been proposed that proinflammatory cytokines, above all those involved in TH1 cell mediated immunity, might be involved in the mechanisms that divert iron from the serum and lead to its accumulation in the reticulo-endothelial system.[57]

The regulation of iron cellular metabolism appears to partly depend on the intracellular level of nitric oxide (NO).[68] In turn NO production is critically dependent upon the levels of the enzyme NO synthase (NOS):[69] the inducible form of NOS (iNOS) may be triggered in several cell lineages by the stimulation of a number of TH1-type cytokines like IL1, TNFα, IFNγ.[69,70]

On these bases, the following sequence of events has been proposed: macrophage activation by IFNγ and TNFα → iNOS expression → NO levels increase → iron storage within the cell. NO exerts its control over intracellular iron homeostasis by regulating the iron regulatory proteins (IRP) 1 and 2.[71,72] These cytoplasmic proteins interact with RNA stem loop structures defined iron responsive elements (IRE) present at the mRNA level of two key iron proteins, ferritin and transferrin receptor, and cause opposite effects on their translation.[73,74] The affinity of IRP to IRE is controlled by the intracellular concentration of iron[73,74] and to some extent by the availability of NO.[57] Thus iron deprivation (or high NO) cause high affinity IRP which induce repression of ferritin mRNA and stabilization of transferrin receptor mRNA. The opposite occurs in conditions of iron overload (or low NO). However, it has been shown that the expression of iNOS itself is regulated by iron and thus increased iron uptake could reduce NOS transcription and NO formation.[68] This autoregulatory loop between iron metabolism and NO pathway, although demonstrated primarily in human and murine cell lines,[68] could represent a central mechanism responsible of the altered macrophage iron traffic in systemic autoimmune disorders and of the development of ACD.[57]

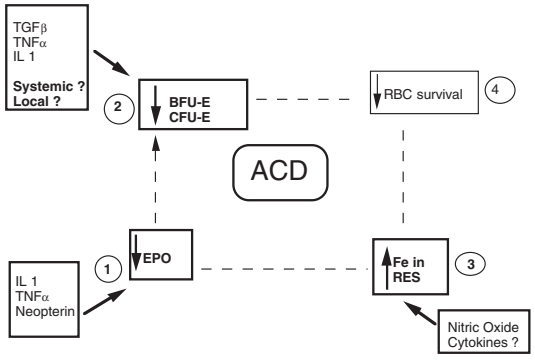
In rat hepatocytes, IL-6 has been shown to promote an increased transferrin uptake by its cell receptor as well as an increased ferritin synthesis independently of the changes of intracellular iron pool.[75] Conceivably, macrophages and hepatocytes have significant differences in their modalities of iron handling. Still, these data indicate that regulatory factors other than IRP may be operating under the influence of proinflammatory cytokines which in macrophages might be able to add further iron to an already saturated intracellular system. Alternatively, a reduced availability of IRP has been hypothesized as a cause of increased uptake and storage of iron.[76]

The possibility that cell-mediated immunity may influence iron metabolism and NO synthesis in systemic autoimmune diseases is strenghtened by two sets of observations. Neopterin, an index of activation of cell-mediated immunity that reflects the activity of the TH-1-type cytokine IFNγ, has been shown to inversely correlate with iron and Hb levels in ACD.[77] TH1-type cytokines stimulate and TH2-type cytokines inhibit iNOS production.[70] These findings are well in keeping with the observation that, in a number of systemic autoimmune disorders frequently accompanied by ACD, like RA, Crohn disease and ulcerative colitis, a prominent pathogenetic role of TH1-type lymphocytes has been documented.[78]

Therapy

Different mechanisms may prevail in the ACD of different autoimmune disorders, even if a cytokine-mediated common rule can be envisaged in the vast majority of cases (Figure 2). Irrespective of the mechanism(s) that control the pathogenesis of ACD, it has become apparent that ACD represents a useful parameter of disease activity.[19] The tenet of ACD treatment is to treat the underlying chronic disorder.[79] If this treatment does not significantly affect the degree of anemia, iron therapy is usually utilized on the ground that ACD may be accompanied by a sideropenic condition which is not easily demonstrable. Actually, ferritin levels may be increased in active systemic autoimmune diseases with ACD simply because hyperferritinemia is part of the spectrum of acute phase response. The only systemic autoimmune disease where intravenous iron therapy has been successfully used to treat ACD in SOJCA.[45,80] It is not unreasonable to suggest that such an approach to ACD may be unique to SOJCA, as these patients also show a number of features fully compatible with a true sideropenia like significant microcytosis with high levels of sTfR and adequate EPO levels.

Subcutaneous EPO has been used in a number of autoimmune disorders with ACD, like RA and autoimmune diseases of the gut.[81-83] The real neces-



1. reduced, inappropriate production of epo
2. reduced growth of erythroid precursors
3. iron accumulation within reticuloendothelial system (res)
4. red blood cell (rbc) reduced life span

Figure 2. Pathophysiology of ACD.

sity of an EPO therapy[84] in systemic autoimmune disorders is usually questionable, because of the rather modest degree of anemia and the high cost of the treatment. If deemed necessary, possible candidates for this type of treatment can be selected taking into account two potentially predictive factors: a) a low basal level of EPO which has proved useful in planning EPO treatment in malignancies;[85-87] b) a high level of proinflammatory cytokines to exclude patients who would need sovramaximal dosage of EPO.[62]

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