

REVIEW ARTICLE**Progress in Understanding the Pathogenesis of the Anemia of Chronic Disease**

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THE ANEMIA of chronic disease (ACD) is usually defined as the anemia occurring in chronic infectious, inflammatory disorders, or neoplastic disorders that is not due to marrow replacement by tumor, bleeding, or hemolysis, and that is characterized by hypoferrremia in the presence of adequate iron stores.^{1,2} ACD does not include the anemias caused by endocrine, renal, or hepatic insufficiency.² Typically, ACD is a mild-to-moderate, normochromic/normocytic anemia, and is characterized by a decreased serum iron and total iron-binding capacity, with normal or increased iron stores demonstrated by serum ferritin or by Prussian blue stain for marrow iron.^{2,3} Reticulocytes are not increased appropriately for the degree of anemia, indicating that this is principally an "underproduction" anemia.

Some investigators take issue with the term "ACD,"⁴ and it may be cogently argued that it is inappropriate because the syndrome includes anemias associated with diseases that are not always chronic, and may not include anemias associated with diseases that are chronic.¹ More accurate terms, such as "sideropenic anemia with reticuloendothelial siderosis,"³ never seem to have become common for obvious reasons. However, despite deficiencies in nomenclature, ACD is an easily recognized clinical entity and may be second only to iron deficiency as a common cause of anemia.² Cash and Sears⁵ reviewed the charts of all anemic patients admitted to the medical service of a metropolitan public hospital over two 2-month periods. Of the 172 anemic patients in whom bleeding, hemolytic disorders, and hematologic malignancies were excluded, 90 (52%) met laboratory criteria for ACD.⁵

Although it is frequently diagnosed, the pathogenesis of ACD has remained unclear. Cartwright³ postulated that at least three pathologic processes were involved in ACD: shortened erythrocyte survival, failure of the bone marrow to increase red blood cell (RBC) production to compensate for this increased demand, and impaired release of iron from the reticuloendothelial system.³ However, the origin of these processes and their relative importance in ACD remain topics for debate.

Cartwright also suggested that the supply of erythropoietin (EPO) to the marrow might be the rate-limiting factor in the impaired marrow response to ACD.³ EPO is the hormone primarily responsible for the regulation of erythropoiesis⁶ and, in ACD, it is inversely correlated with the

hemoglobin: as the hemoglobin decreases, the EPO level increases.⁷ Because of its primary importance in erythropoiesis, EPO has been a major focus of investigation in ACD.

The anemia associated with rheumatoid arthritis (RA) has often served as a model for ACD, and the initial investigations of EPO levels in ACD were performed in patients with RA. Although these patients increased their serum EPO in response to the development of anemia,⁸ the EPO levels attained were lower than those detected in equally anemic patients without RA.^{9,10} Baer et al¹¹ compared the EPO response to anemia of 54 RA patients to that of 41 patients without RA. Both groups of patients showed a linear inverse correlation between the log of the serum EPO level and the hemoglobin concentration, but the line for the RA patients was shifted downward, indicating a blunted EPO response to anemia in RA patients.¹¹ This finding has been confirmed by other investigators¹² who subsequently demonstrated similar results in patients with cancer¹³ and acquired immunodeficiency syndrome (AIDS).¹⁴

While the decrease in the incremental response of EPO to anemia may contribute to the reduced erythropoiesis in ACD, it cannot be considered the primary cause because EPO levels are still higher than those seen in persons without anemia. The failure of the bone marrow to respond to these increases in EPO must be considered the primary reason for the anemia.

Recently some advances have been made in the understanding of the pathogenesis of ACD, particularly in regard to the role of cytokines as initiators and mediators of this anemia.^{15,16} We propose to review these advances here.

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INHIBITION OF ERYTHROPOIESIS IN ACD

Many investigators have demonstrated cellular or humoral inhibition of erythropoiesis in ACD. Zanjani et al¹⁷ found that erythroid colony formation in vitro in a subset of patients with disseminated histoplasmosis was enhanced by depletion of macrophages, while macrophage depletion of normal marrow did not affect colony formation. Addition of macrophages from these patients to macrophage-depleted normal marrow inhibited colony formation, while macrophages from normal individuals did not inhibit colony formation by patient marrow. This macrophage-dependent inhibition resolved with response of the infection to therapy.¹⁷ Roodman et al¹⁸ reported similar findings in other patients with ACD. In contrast, Reid et al¹⁹ did not find inhibition of erythroid colony formation in vitro by adherent cells from anemic patients with RA, but did report that serum from these patients inhibited colony formation, while serum from nonanemic RA patients or from normals had no inhibitory effect.¹⁹ Dainiak et al²⁰ also reported an inhibitory effect of serum from patients with RA or systemic lupus erythematosus. Baer et al²¹ found suppression of erythroid colony-forming units (CFU-E) in vitro by serum from two of five anemic patients with RA but also by serum from one of four nonanemic RA patients, demonstrating the variability of results that complicates studies requiring addition of whole serum. Cells other than adherent cells also have been implicated as mediators of ACD, because Sugimoto et al²² reported inhibition of erythroid colony formation by peripheral blood T lymphocytes (but not by serum) from anemic RA patients.

Inhibition of Erythropoiesis by Specific Cytokines

Improved understanding of the pathogenesis of inflammation has led to the identification of cytokines that are involved in this process, and these cytokines have become available in highly purified or recombinant preparations. Several cytokines involved in the inflammatory response are increased in the diseases associated with ACD, and have been used to develop models for this syndrome. This has allowed more precise delineation of the mechanisms by which erythropoiesis is inhibited in ACD.

Tumor necrosis factor α (TNF). TNF plays a significant role in inflammation and the immune response.²³ TNF levels have been reported to be increased in patients with cancer,^{24,25} RA,²⁵ parasitic and bacterial infections^{25,26} (Fig 1), as well as in patients with the AIDS and AIDS-related complex.²⁷

Chronic administration of TNF to animals (either by intermittent injection or by implantation of TNF-producing cells) resulted in the development of anemia.²⁸⁻³¹ No significant decreases in platelet or granulocyte counts were noted.^{28,29} Like ACD in humans, this anemia was associated with a low serum iron and normal iron stores.^{30,31} Mice exposed to TNF as a single intravenous (IV) dose showed suppression of spleen and marrow CFU-E but increased numbers of colonies from erythroid burst-forming units (BFU-E) and granulocyte-macrophage colony-forming units (CFU-GM),²⁹ while mice into which TNF-producing Chi-

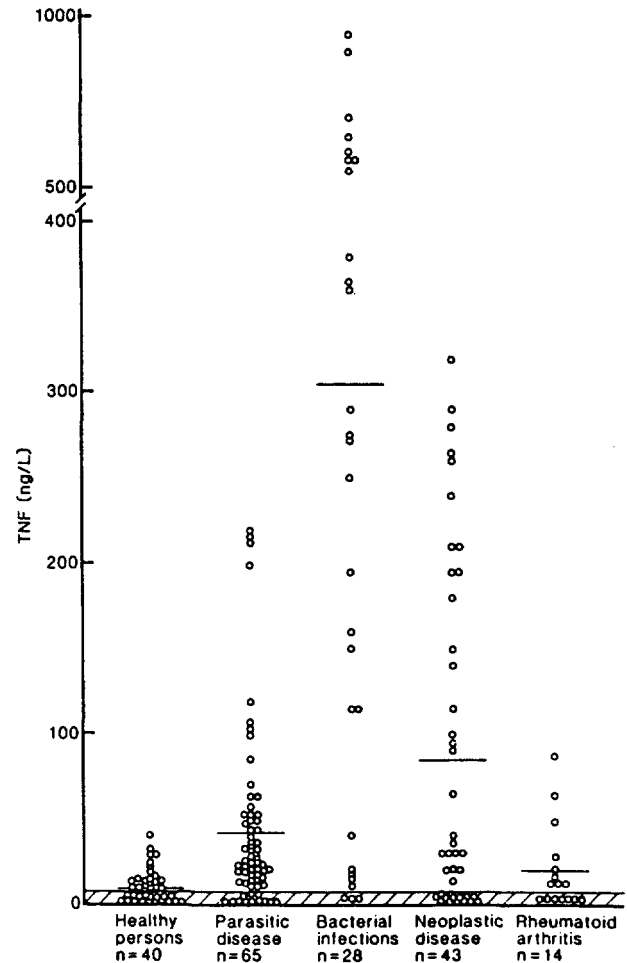


Fig 1. TNF concentrations in healthy and diseased persons. Dotted area represents values below the detection limit of the method. Horizontal lines represent mean values of TNF concentrations for each group. Reprinted with permission.²⁵

nese hamster ovary cells had been injected showed suppression of BFU-E as well as CFU-E.³⁰ As discussed below, TNF appears to mediate the interleukin-1 (IL-1)-induced inhibition of erythropoiesis in the in vivo murine model of Johnson et al.^{32,33}

Recombinant TNF was administered to patients with metastatic cancer in a phase I trial.³⁴ They received 1 to 250 $\mu\text{g}/\text{m}^2$ TNF twice weekly for 4 weeks. The 16 patients who completed 4 weeks of therapy became anemic, with the mean hemoglobin decreasing from 118 g/L to 92 g/L, while no significant decreases in granulocytes or platelets were noted.³⁴ In vitro inhibition of human erythroid colony formation (BFU-E and CFU-E) by TNF has also been demonstrated,^{35,36} as has selective inhibition of late erythroid progenitors (CFU-E) relative to late granulocytic progenitors (CFU-G).³⁷ Roodman et al³⁵ suggested, based on studies of plucked BFU-E colonies, that TNF directly inhibited these human erythroid progenitors. However, the capacity to generate highly purified human CFU-E from peripheral blood BFU-E³⁸ allowed Means et al³⁹ to demonstrate that the inhibitory effect of TNF on CFU-E colony

formation was indirect and mediated by a soluble factor released from marrow stromal cells. This factor has recently been identified as β interferon (β IFN),⁴⁰ which is produced by marrow stromal cells in response to superinduction with poly I-poly C⁴¹ and, presumably, in response to other inducers of the immune response. These studies indicate that while TNF may act directly on BFU-E, its effect on CFU-E colony formation is indirect and is mediated by β IFN released from marrow stroma in response to TNF.

IL-1. IL-1 is a polypeptide that has a wide variety of actions in inflammation and immunity⁴² and shares many of the properties of TNF.⁴³ Levels of IL-1 are elevated in patients with RA (as well as other ACD-associated conditions,^{44,45} and this elevation correlates with markers of disease activity, such as anemia.^{46,47}

IL-1 has also been shown to inhibit murine erythropoiesis in vitro⁴⁸ and in vivo.³² Johnson et al³² treated mice with single or repeated intraperitoneal injections of recombinant human IL-1 α .³² At 6 hours postinjection, significant suppression of mature erythroid progenitors (CFU-E) occurred, reaching a maximum at 24 hours. This coincided with suppression of the peripheral blood reticulocyte count to 25% of the pretreatment value.³² Less mature erythroid progenitors (BFU-E), as well as granulocytic, monocytic, and megakaryocytic progenitors, were stimulated by IL-1 α , with the maximum effect seen at 48 hours. After repeated injections of IL-1 α , the mice became anemic. In a subsequent study using the same murine model, Furmanski and Johnson³³ reported that the inhibitory effect of IL-1 α is mediated by TNF α .

IL-1 has also been implicated in ACD in humans. Maury et al⁴⁶ have reported that IL-1 β levels are slightly elevated in RA patients relative to controls, and are significantly elevated in anemic RA patients relative to RA patients who are not anemic (Fig 2). Other investigators⁴⁷ have shown that the decrease in the hemoglobin levels of RA patients is directly correlated with the IL-1 level. Maury et al⁴⁶ also showed that recombinant human IL-1 (α and β) inhibited in vitro colony formation by BFU-E and CFU-E from normal human marrow, as well as proliferation by human erythroleukemia cells. In vitro colony formation by marrow granulocyte-macrophage progenitors (CFU-GM) was not inhibited by IL-1.⁴⁶

Means et al⁴⁹ investigated the inhibition of human CFU-E colony formation by recombinant human IL-1 β (rhIL-1) and found that rhIL-1 inhibited colony formation by unpurified marrow CFU-E but not by highly purified CFU-E, indicating that this inhibitory effect was indirect (Fig 3). Further studies showed that this inhibitory effect required the presence of T lymphocytes, and was mediated by γ interferon (γ IFN). rh γ IFN, in turn, directly inhibited CFU-E colony formation (Fig 4).⁴⁹ This correlated well with data from other investigators (reviewed below) implicating γ IFN in ACD. Because γ IFN inhibits colony formation by CFU-GM^{50,51} as well as by erythroid progenitors, this result would appear initially to be in conflict with the erythroid specificity of inhibition by IL-1 reported by Maury et al.⁴⁶ However, IL-1 also leads to the release of granulocyte-macrophage (GM) and granulocyte (G) colony-

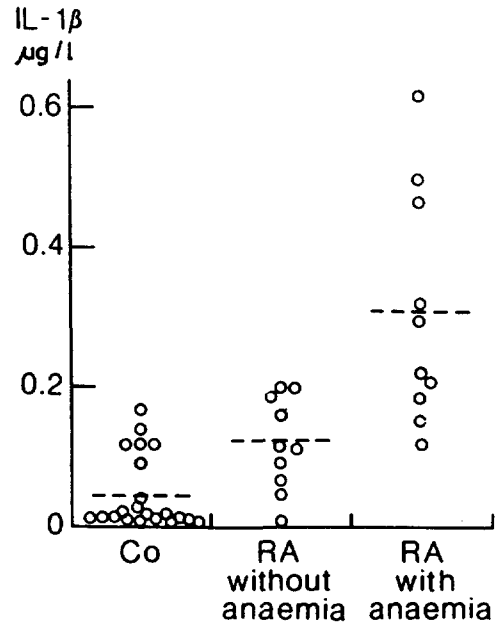


Fig 2. Serum IL-1 β concentrations in blood donors (Co) and in patients with rheumatoid arthritis (RA) with and without anemia. The horizontal line indicates the mean concentration. The difference between the anaemic and nonanaemic patients with RA is significant ($P < .01$). Reprinted with permission.⁴⁶

stimulating factors (CSFs),⁵² which can overcome the inhibitory effects of γ IFN on myeloid progenitors.⁵³ CFU-E colony formation, which is not effected by G- or GM-CSF,⁵⁴ would not be amenable to "rescue" from inhibition by these growth factors.⁴⁹

γ IFN. γ IFN is produced mainly by T lymphocytes and is involved in the modulation of immune and inflammatory responses as well as the host defense against microbial challenge.^{55,56} Elevated γ IFN levels have been reported in

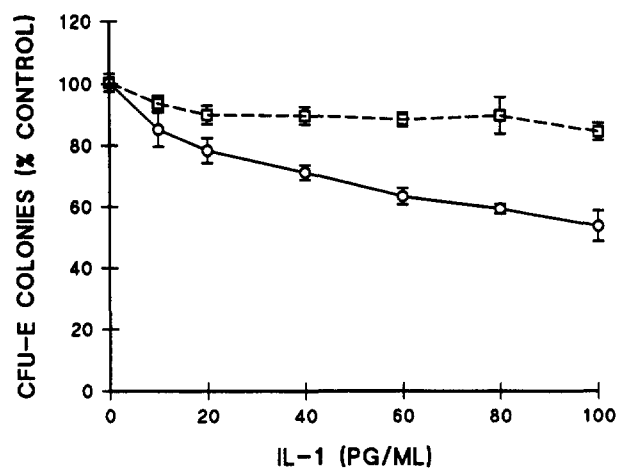


Fig 3. Effect of rhIL-1 on CFU-E colony formation by blood BFU-E derived CFU-E and marrow CFU-E. Growth of highly purified CFU-E colonies from three experiments ($44.4 \pm 32.4\%$ CFU-E) (\square) and marrow CFU-E colonies from three experiments ($0.36 \pm 0.09\%$ CFU-E) (\circ). Data from each experiment were normalized to CFU-E colony growth without IL-1. Results are expressed as mean \pm SE. Reprinted with permission.⁴⁹

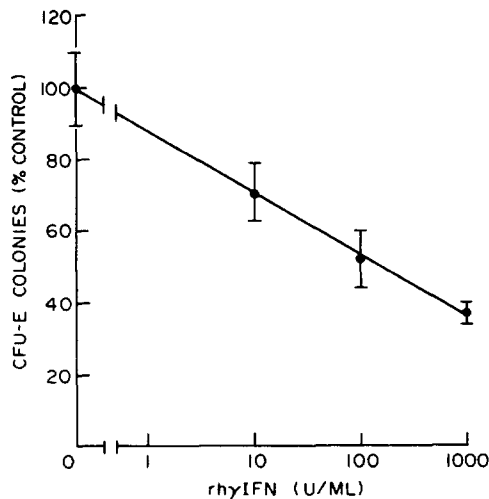


Fig 4. Effects of rh γ IFN on highly purified CFU-E colony formation. Results of three experiments with CFU-E purity $24.0\% \pm 3.7\%$ are combined. Data from each experiment were normalized to CFU-E colony growth without rh γ IFN. Results are expressed as mean \pm SE. Reprinted with permission.⁴⁹

patients with autoimmune and infectious diseases,^{55,57} and cancer patients treated with γ IFN may develop a normochromic/normocytic anemia.⁵⁸ γ IFN has also been implicated in the pathogenesis of aplastic anemia.^{51,59-61}

A number of investigators have reported inhibition of human erythroid colony formation in vitro by γ IFN.^{49,51,62,63} Mamus et al,⁶² looking at both BFU-E and CFU-E colony formation, reported that the inhibitory effect of γ IFN was indirect and required accessory cells, while Raefsky et al⁶³ and Means et al,⁴⁹ studying colony formation from purified progenitors, reported that this inhibitory effect was the result of direct action of γ IFN on the CFU-E.

Denz et al⁶⁴ have investigated the correlation between anemia and markers of immune activation such as γ IFN and neopterin. Neopterin is a pteridine that indicates activation of macrophages by γ IFN, and the neopterin level is increased in a variety of infectious, inflammatory, and malignant disorders.^{16,65,66} Denz et al⁶⁴ studied 25 patients with hematologic malignancies, 44% of whom were anemic (hemoglobin < 12 g/dL). Serum neopterin levels showed a significant inverse correlation with hemoglobin. Neopterin and γ IFN levels, in turn, showed a significant direct correlation,⁶⁴ demonstrating a relationship between anemia and a marker and mediator of immune activation, as Maury et al⁴⁶ and Eastgate et al⁴⁷ had previously shown for IL-1.

Other cytokines. Many cytokines involved in the inflammatory and immune response inhibit erythroid colony formation in vitro or are associated with development of anemia, including α and β IFNs,^{40,63,67} IL-6,⁶⁸ transforming growth factor- β (TGF β),^{69,70} and may merit investigation for a role in the pathogenesis of ACD. Recent data from two groups suggests that α IFN, though a direct inhibitor of BFU-E, acts indirectly through accessory cells to inhibit CFU-E colony formation.^{71,72} β IFN appears to directly inhibit CFU-E colony formation.⁴⁰

IL-6 is an attractive candidate as a mediator of ACD

because its production is increased in inflammatory arthritides such as RA,⁷³ and its administration to primates results in anemia.⁶⁸ Vreugdenhil et al⁷⁴ demonstrated elevated serum IL-6 levels in RA patients with ACD relative to nonanemic RA patients, and showed that these levels correlated with markers of disease activity such as the sedimentation rate. However, the addition of IL-6 to bone marrow erythroid progenitors in vitro did not inhibit erythroid colony formation. In fact, the addition of anti-IL-6 impaired in vitro erythropoiesis, suggesting that the effect of IL-6 was stimulatory rather than inhibitory. The investigators concluded that IL-6 was a marker of RA activity, but played no role in ACD.⁷⁴

TGF β produces anemia when injected into mice⁷⁵ and inhibits colony formation by IL-3-dependent progenitors such as BFU-E,⁷⁰ but does not inhibit colony formation by CFU-E.⁷⁶

Synergy between cytokines. In addition to their own effects, cytokines implicated in the pathogenesis of ACD can exhibit synergy. Synergistic inhibition of erythroid progenitors in vitro by α IFN and γ IFN has been reported by Raefsky et al⁶³ and by Means and Krantz.⁷¹ γ IFN also shows synergy with TNF in its inhibitory effect on hematopoiesis in vitro.⁷⁷ In addition, various cytokines involved in the inflammatory response have amplification pathways. TNF and IL-1 each induce expression of the other cytokine, and also increase their own expression.^{23,42,44,78}

CYTOKINE INHIBITION OF EPO PRODUCTION AND EPO ACTION

Mechanism of Impaired EPO Response To Anemia

As noted above, the EPO response to anemia is blunted in ACD, and this relative EPO deficiency, while not the primary mechanism for decreased erythropoiesis, may contribute to the development of ACD. Recent investigations have suggested a role for cytokines in the impaired EPO response associated with RA. Faquin et al⁷⁹ reported that IL-1 (α or β), TNF α , and TGF β inhibited production of EPO from the hepatoma cell line Hep3B. This effect appeared to occur at the level of the EPO mRNA.⁷⁹ Jelkmann et al,⁸⁰ using the HepG2 line, reported similar results for IL-1 and TNF, but noted no inhibition by TGF β . In addition, they reported that IL-1 β inhibited EPO production in isolated serum-free perfused rat kidneys.⁸⁰ So by inhibiting EPO production as well as marrow erythropoiesis, cytokines such as IL-1 and TNF may amplify their contributions to the development of ACD.

Cytokines and Impaired EPO Action

The relative deficiency in the EPO response in ACD led to consideration of the use of EPO as treatment for this condition. Gutnisky and Van Dyke⁸¹ showed that the anemia induced in rats by turpentine abscesses could be corrected by injection of human urinary EPO, indicating that ACD could respond to EPO. The anemia associated with adjuvant-induced arthritis in rats also responded to EPO.⁸² When rhEPO became available, studies were performed which demonstrated that pharmacologic concentra-

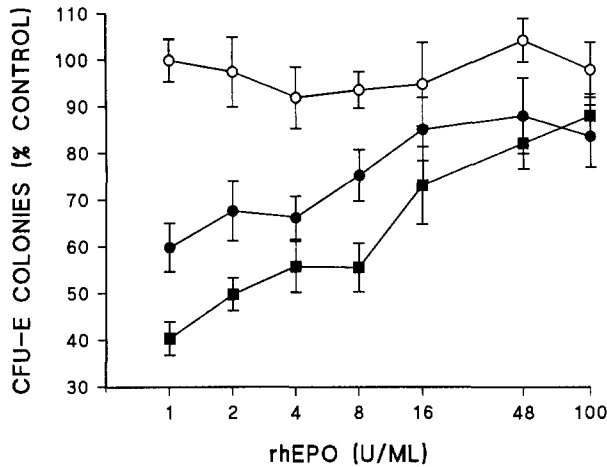


Fig 5. Effect of varying EPO concentrations on CFU-E colony formation by mononuclear marrow cells in the presence of recombinant human γ IFN ([○], γ IFN 0 U/mL; [●], γ IFN 100 U/mL; [■], γ IFN 1,000 U/mL). 100% control is defined as CFU-E colony formation in the presence of 1 U/mL recombinant human EPO (rhEPO) and 0 U/mL γ IFN. Each point represents data from three separate experiments with 290 ± 30 CFU-E/ 10^5 cells. Modified with permission.⁹³

tions of EPO corrected the anemia in patients with RA,⁸³⁻⁸⁶ cancer,⁸⁷⁻⁸⁹ and AIDS.⁹⁰ Sixteen anemic patients with RA were treated with EPO in a multicenter trial.⁸⁴ Twelve responded with an increase in hematocrit of 6 points, and 11 of these patients normalized their hematocrits.⁸⁴

EPO can also correct the inhibition of erythropoiesis in some of the cytokine-based models of ACD discussed above. Administration of EPO corrects the suppression of CFU-E seen in mice injected with IL-1,³² as well as the anemia and reduction in CFU-E numbers reported in mice treated with single injections of TNF.⁹¹ However, it does not correct the anemia or reduction in erythroid progenitors in mice exposed continuously to TNF *in vivo*, which may reflect the relatively higher concentrations of TNF in these animals.⁹²

The inhibition of *in vitro* colony formation by either highly purified human CFU-E generated from peripheral blood BFU-E or by human marrow CFU-E induced by γ IFN was also corrected by exposure to very high, pharmacologic, concentrations of EPO.⁹³ Normally, CFU-E colony formation is not increased by EPO levels higher than 1 U/mL,³⁸ which saturates the 1,000 EPO receptors present on each CFU-E.⁹⁴ However, in the presence of γ IFN concentrations from 100 to 1,000 U/mL, 16 to 64 U/mL EPO was necessary to restore CFU-E colony formation to baseline levels⁹³ (Fig 5). The amount of EPO required to overcome the inhibitory effect depended on the amount of γ IFN present, suggesting that cases of ACD not responsive to EPO may have extremely high levels of cytokine inhibitors of erythropoiesis (such as γ IFN or TNF) that would require EPO levels higher than can be pharmacologically attained for improvement of erythropoiesis. Whether γ IFN might be decreasing the binding of EPO to its receptors, or the reverse, has not yet been determined, but the means are now available to determine the precise mechanism by which

a cytokine such as γ IFN inhibits the action of EPO on erythroid progenitor cells.

CYTOKINES AND ALTERED IRON METABOLISM IN ACD

The distinctive feature of ACD is a low serum iron in the presence of adequate reticuloendothelial (RE) iron stores.³ This has prompted extensive investigation of iron metabolism in ACD. Early studies demonstrated a block in release of RE iron, and implied that ACD involved a "functional" iron deficiency.^{95,96} However, other investigators, using lower doses of radiolabeled iron, found no block in iron reutilization or mobilization, suggesting that the normal RE iron stores with hypoferrremia is a secondary phenomenon caused by decreased erythropoiesis.⁹⁷⁻¹⁰⁰

Recent studies of the role of cytokines in ACD suggest that both impaired iron metabolism and impaired erythropoiesis are involved in ACD. Impairment of erythropoiesis, either by inhibition of progenitors or reduction of EPO increment, has been reviewed above. Moldawer et al³¹ and Alvarez-Hernandez et al¹⁰¹ injected rats and mice, respectively, with recombinant TNF and induced anemia and hypoferrremia. The hypoferrremia was associated with abnormalities of iron release from the RE system and incorporation into RBCs.^{31,101} In addition, Denz et al¹⁰² have reported a correlation between neopterin (a marker of immune activation associated with anemia in ACD patients and with γ IFN production⁶⁴⁻⁶⁶ and ferritin levels in patients with malignancies, suggesting a role for immune activation in altered iron metabolism. Finally, Rogers et al¹⁰³ have recently reported that IL-1 increases ferritin production, and suggested that this additional ferritin could act as a trap for iron that might otherwise be available for erythropoiesis. While impairment of iron metabolism and erythropoiesis are each present in ACD, several reasons exist for believing that the latter is the most important contributor to the anemia. First, EPO can correct anemia in ACD (reviewed above) but cannot overcome the anemia of iron deficiency.^{104,105} If relative iron deficiency was the primary cause of ACD, treatment with EPO would not correct this anemia. Secondly, in a multicenter trial of EPO in anemic

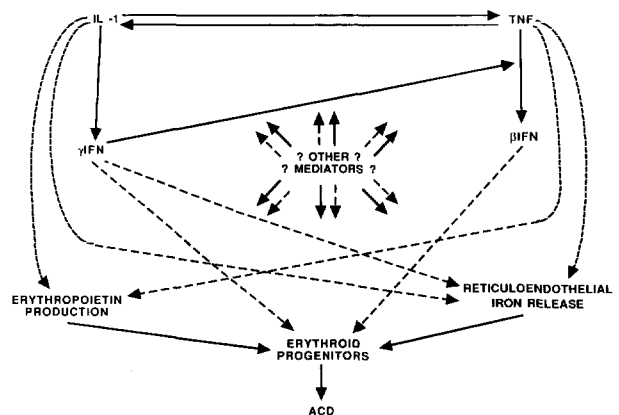


Fig 6. Schematic diagram representing effects of inflammatory cytokines on processes involved in the impairment of erythropoiesis in ACD. Positive regulatory effects are indicated by solid lines and negative effects by broken lines.

RA patients, administration of oral iron to eight iron-deficient RA patients increased serum ferritin levels but did not significantly increase their hematocrits, while EPO normalized the hematocrits of 11 of 12 patients (who also received concomitant iron).⁸⁴ Finally, serum levels of transferrin receptor are increased in 15 of 17 patients with iron deficiency, but are only increased in 4 of 41 patients with ACD.¹⁰⁶

SUMMARY

Improved understanding of the inflammatory response and the identification and characterization of the specific cytokines involved, as well as improved understanding of erythropoiesis, and the availability of recombinant human growth factors such as EPO, have greatly enhanced our appreciation of the pathogenesis of ACD by allowing

development of a number of informative models for studying this syndrome. It appears that a variety of cytokines are involved in all aspects of the pathogenesis of ACD, from the inhibition of erythroid progenitors and EPO production to impairment of iron release. A schematic of the contributions of some of these cytokines to the development of ACD is shown in Fig 6. The exact biochemical mechanisms by which these effects occur is still to be determined.

The progress outlined in this report has allowed us to develop a more precise understanding of the pathogenesis of this common and important clinical syndrome. In 1983, Hansen¹ subtitled a review of ACD "A Bag of Unsolved Questions." Although this description is still accurate, our understanding of ACD has now developed to the point where we can offer a more defined subtitle: "A Bag of Cytokines."

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