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The data on the relationship between iron deficiency and infection are conflicting. Some researchers conclude that mild iron deficiency is beneficial for immunity, whereas others contend that any deficit is not good for immunity. Additionally, infection or inflammation generate anemia and profound changes in iron metabolism mediated by cytokines. These changes are important confounders to consider in assessments of iron status.

Introduction

The interaction between host and infectious agent is a complex phenomenon, and no theory or experimental model fully explains it. The central focus of scientific inquiry should be to identify the basic processes and factors of both the human immune response and infectious agent virulence. The literature on the relationship between iron deficiency and infection contains conflicting data and divergent results. Some investigators favor the contention that mild iron deficiency is beneficial for immunity, whereas others contend that any iron deficit is not good for immunity. In addition, infection or inflammation generate anemia (the anemia of chronic infection) and profound changes in iron metabolism mediated by cytokines. The study of these mediators of the immune response aid in understanding the relationship between anemia, iron, and infection. The changes induced by infection and inflammation on iron metabolism are important confounders to be considered when individuals or surveys are assessed for iron status.

This paper reviews clinical and epidemiologic studies of iron status and infection, the effects of infection on iron metabolism and anemia, and the effects of iron deficiency anemia on immune function.

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Clinical Studies on the Relation Between Iron Status and Infection

Evidence That Iron May Promote Infection

More than 100 years ago, Trousseau¹ observed that iron supplementation of patients with quiescent tuberculosis often led to clinical recurrence. McFarlane et al.² suggested that the rapid deterioration and death of infants with kwashiorkor was related to refeeding, especially micronutrient supplements containing iron. A direct correlation between serum transferrin concentration and survival was observed in a group of 40 children treated with a high-protein diet, antimalarial agents, vitamins, and iron. Overwhelming infection was the most frequent cause of death. After 2 weeks of treatment, the mean serum transferrin concentration of those who survived was 130 mg/dL versus 30 mg/dL in those who did not survive. Serum albumin concentrations were also lower in those who died. Serum from infants dying of infection supported the growth of *Staphylococcus aureus*, whereas the addition of purified transferrin to the cultures inhibited bacterial growth. McFarlane and coworkers suggested that in patients with low serum transferrin concentration, iron therapy resulted in a high transferrin saturation that promoted bacterial infection.³ However, another explanation for these findings is that those who died had more severe kwashiorkor, as indicated by low serum transferrin and albumin concentrations.

Studies by Murray et al.⁴⁻⁶ are widely quoted as evidence for a protective effect of iron deficiency on infection. In a prospective randomized trial of 137 adult Somali nomads with iron deficiency anemia, only patients with an otherwise normal nutritional status were enrolled. These subjects were given 900 mg of oral ferrous sulfate or a placebo for 1 month. Iron treatment raised serum iron and hemoglobin concentrations. In the untreated group ($n=71$), there were 3 episodes (7.6%) of infection, compared with 36 episodes in 27 subjects (38%) in the iron-supplemented group ($n=66$). Differences in rates of infection were noted for malaria, brucellosis, and tuberculosis. Although this study has been criticized because the follow-up time was limited and the study was not double-blinded, it is the most convincing evidence that oral iron treatment may increase the incidence of certain infectious illnesses.

Evidence That Iron May Protect Against Infection

In 1928, Mackay reported the results of a survey of 154 nonhospitalized infants in London.⁷ She observed that anemia was common in breast-fed and cow's milk-fed infants. Oral iron supplementation not only raised hemoglobin, but also allegedly reduced the incidence of respiratory and diarrheal disease by 50% compared with untreated controls. Unfortunately, important intervening variables were not reported. Furthermore, the study compared successive years of treated and untreated populations instead of concurrently treated and untreated groups. However, it is remarkable that Mackay recommended 60 years ago that formula-fed infants be given an iron supplement before 2 months of age and observed that many breast-fed infants also require iron treatment. Little has changed since.

Thirty years ago, Andelman and Sered⁸ described the effect of feeding an iron-fortified formula for 6 to 9 months to 603 infants of low socioeconomic status in Chicago and compared the results with 445 control infants fed a non-iron-fortified evaporated milk formula. Although growth was similar in both groups, 9% of the iron-treated infants had anemia compared with 76% of the untreated infants. There was also a striking reduction in the incidence of respiratory infection in the group receiving iron-fortified formula. This study has been criticized for the loose criteria defining infection and for the dependence on parental recall of illness. The same criticisms apply to a study by Burman⁹ in which infants 3 to 24 months old were randomized to receive iron or no supplementation, with no difference in infection found between groups.

Lovic¹⁰ found that anemic children had a significantly higher prevalence of gastroenteritis than nonanemic controls. However, it is unclear whether the gastroenteritis was the cause or the consequence of the anemia. Another study of Maori infants showed that infants who received parenteral iron dextran in the neonatal period had lower hospital admission rates during the subsequent 2 years, principally for respiratory and gastrointestinal infection, than untreated controls.¹¹ Randomization of infants and the selection of clinical endpoints were probably inadequate. Hospital admissions are not always based on standardized criteria, and these in turn may change over time.

Oppenheimer et al.¹² showed in a retrospective study that meningitis and pneumonia were more common in the presence of iron deficiency in hospitalized infants in Papua New Guinea. However, the effect of infection on iron status measurements is now known to be a confounding factor in attempts to establish a relationship between iron status and

infection after infection has occurred. The knowledge that alterations in iron status, which mimic iron deficiency anemia, may last several days or weeks after an acute infectious episode will be reviewed below.

Parenteral Iron Therapy

Barry and Reeve¹³ reported the results of a study in which a large number of Polynesian neonates were given prophylactic intramuscular iron dextran. During a 2-year period, the incidence of neonatal sepsis (usually caused by *E. coli*) was 22 per 1000 infants. After discontinuing the administration of iron dextran, the incidence of sepsis decreased to 1.8 per 1000 infants. Most infections occurred 4 to 10 days after injection without evidence of localized infection at the injection site.

Several flaws in this study discredit the author's conclusion that iron treatment was related to the increased incidence of death from sepsis. Rates of infection before the use of iron dextran were not provided, and because the entire population was treated, there were no simultaneous controls. It is also not clear whether the iron itself or the parenteral iron dextran preparation was responsible for the effect. Unfortunately, once this paper was published, ethical issues hindered the study of the use of parenteral iron in neonates; thus, a well-planned study could not be carried out to clarify this issue.

In contrast to the study of Barry and Reeve, no increase in susceptibility to infection was seen in a U.S. study in which premature infants received prophylactic iron dextran.¹⁴ Also, a Finnish study of premature infants showed markedly lower infection rates during the first 6 months of life in neonates given prophylactic iron dextran than in untreated controls.¹⁵ Additionally, before Barry and Reeve's study was published, we gave 150 mg of iron dextran to 500 newborns in a maternity hospital in Santiago, and no cases of severe infection were detected during the first 4 days of life. Unfortunately, because 10 infants were lost to follow-up, the data on infection rates were not published (M. Olivares, personal communication).

More recently, an extensive and carefully designed prospective, double-blind, longitudinal protocol was carried out by Oppenheimer and coworkers in Madang, Papua New Guinea, where malaria is endemic.¹⁶ A total of 486 newborn infants were randomized to receive either 150 mg of elemental iron as intramuscular iron dextran or a placebo at 2 months of age. After 12 months of follow-up, death rates were similar in both groups, with lower respiratory infection related to measles or pertussis the primary cause of death. However, in the iron-treated

group, there was an increased incidence of otitis media, severe lower respiratory infections, malarial parasitemia, and splenomegaly rates. Hospital admissions associated with measles and malaria were also higher. After 6 months, 18.5% of the iron-treated group and 11.3% of controls were positive for malaria; after 12 months, the percentages positive were 33% and 20%, respectively. Nevertheless, no significant difference was found in the degree of parasitemia in the positive subjects.

These carefully designed studies show that the difference in infection rates between iron-treated and non-iron-treated infants is at best marginal, except perhaps for malaria, a chronic disease for which infection rate and disease detectability are not synonymous. In the pathogenesis of malaria, a parasite that infects the red blood cell, newer erythrocytes are more susceptible to infection. Thus, it is conceivable that iron-deficient infants do not have as heavy a burden of parasitemia as do the iron-replete infants and that actual infection rates are similar.

The contradictory findings reported in the literature on the interaction of iron and infection may be due to differences in the degree of exposure to infection. Most reports that support the concept of an increased risk of infection after iron treatment are based on studies of disadvantaged populations in developing tropical countries. In these populations, it is valid to assume that other nutritional deficits in addition to iron deficiency may be a factor in the susceptibility to infection. The only condition that seems to be enhanced by iron supplementation is malaria, probably because of the pathogenesis of this disease. Nevertheless, the data are far from conclusive.

Effect of Iron-fortified Foods on Infection During Infancy

We were able to evaluate data from three field trials in Chile that examined the effect of iron-fortified foods on infection. The Chilean government sponsors the National Supplementary Food Program (NSFP), which provides free milk to children. The program has considerable prestige and acceptance. It provides 3 kg of full-fat milk per month from birth to 6 months of age and 2 kg up to 24 months of age. The program reaches more than 75% of eligible Chilean children. The milk provided by the NSFP is not iron fortified. The children enrolled in these field studies were of middle to low socioeconomic status, living in houses built of solid material, with running water, a sewage system, and electricity.

Study 1. The study population lived in urban Santiago, Chile, in an area served by two outpatient

clinics of the National Health System (NHS). Infants for this study were selected randomly from participants in a larger field trial designed to determine the effects of iron-fortified milk on the iron nutritional status of infants.¹⁷ The larger field trial prospectively followed infants who had received two types of milk from 3 to 15 months of age. The infants were randomly assigned to an iron-fortified milk group ($n=198$) or a non-iron-fortified milk group ($n=184$). The iron-fortified milk group received a full-fat (26%) powdered milk fortified with 15 mg iron as ferrous sulfate, 100 mg of ascorbic acid, 1500 IU of vitamin A, and 400 IU of vitamin D per 100 g powder. The iron-fortified product was slightly acidified (total acidity = 2.5 g lactic acid/L) to discourage its consumption by other members of the family. The non-iron-fortified milk was a nonacidified, nonfortified similar powdered product. Both milks were provided through the clinic, and there was no noticeable difference in milk consumption between groups. Solid foods were introduced to all infants according to the usual practice in Chile: fruits and juices at 2 months, vegetables and meats at 4 months, legumes at 6 months, and table foods at 9 months of age.

Partially or fully weaned 3-month-old infants were considered for inclusion in the morbidity study if they met the following criteria: birth weight > 2500 grams and free of perinatal illness, chronic disease, malnutrition, blood transfusion, and iron therapy. Seventy-four recipients of iron-fortified milk and 76 control infants were enrolled in the study.

Both groups received home visits by a trained field nurse every week, and mothers were instructed to keep a daily record of symptoms and signs. A standardized form was provided to record the following: number and character of the stools (formed, pasty, liquid, mucous, or bloody), cough and/or wheezing with or without fever, and nasal discharge. One episode of diarrhea was defined as the presence of liquid stools for more than 24 hours. One episode of respiratory illness was defined as cough and/or wheezing for a duration of at least 5 days. Second episodes were those occurring after 7 or more symptom-free days. Every 2 weeks, the nurse obtained information on the infant's food intake. Consumption of iron-fortified milk was confirmed by serial determinations of iron in stools.

Infants at 3, 9, and 15 months of age were seen at the clinic, where anthropometric measurements and determinations of iron nutritional status were performed. Blood sampling was delayed for children who were clinically ill at the time of a scheduled clinic visit.

Criteria for exclusion from the study were he-

moglobin levels below 9 g/dL at 9 months of age, failure to follow the protocol, less than 45 completed morbidity questionnaires, a level of iron in stools lower than previously determined as proof of consistent iron-fortified milk intake, and breast-feeding exclusively for more than 120 days. Fifty cases were not evaluated owing to prolonged breast-feeding. This left a total of 53 infants receiving iron-fortified milk and 47 receiving non-iron-fortified milk for data analysis.

The prevalence of anemia and iron deficiency was significantly less in the iron-fortified group at 9 or 12 months of age. There were no differences between the two groups in any of the intervening variables.

The mean number of episodes of diarrhea was 1.1 per year per child in the fortified group and 1.2 per year per child in the nonfortified group. The figure for lower respiratory infections was 3.9 per year per child in both groups. Also, 49.1% of the infants in the fortified group and 38.3% in the nonfortified group never developed diarrhea. The incidence of respiratory infection was 5.7% and 10.6% for the fortified and nonfortified groups, respectively. All differences were not statistically significant.

The main result of this prospective controlled study was to show that iron supplementation of milk at doses sufficient to abolish iron deficiency anemia did not result in a significantly increased incidence of diarrhea or respiratory illness.

Study 2. This regional field trial was conducted to determine whether the results of the first study could be reproduced under the standard operating conditions of the NHS clinics. The premise was that replacement of the non-iron-fortified formula distributed by the NHS with the iron-fortified formula would prevent iron deficiency in the vast majority of Chilean children reached through this program.¹⁸

Two groups of spontaneously weaned infants were studied between June 1978 and February 1980 in all of the NHS clinics in the central area of Santiago. Infants born before July 31, 1978, continued on the regular non-iron-fortified milk program, which consisted of 3 kg full-fat powdered milk per month until the age of 6 months, and 2 kg per month thereafter. As the control group, infants born after August 1, 1978, were given an equivalent amount of acidified iron-fortified milk with ascorbic acid. Health care was identical for the two groups. Detailed medical and nutritional status information was collected for 585 infants born in June and July who received non-iron-fortified milk and for 654 infants born in August and September who received iron-fortified milk. These infants were followed until at least 9 months of age.

At 9 and 15 months of age, laboratory tests of iron status were performed on a subsample of approximately 200 infants in each group. These subsamples were randomly selected from the infants being followed in the seven participating clinics. Infants were selected on the basis of whether they were actually consuming the prescribed milk, without consideration of other demographic factors such as birth weight. Clinic personnel provided well-baby care, took anthropometric measurements, and treated illnesses.

Initially, the general characteristics of the two groups were similar. There were no differences in birth weight, socioeconomic condition, maternal age, or parity. Breast-feeding was actively encouraged at the clinics. The data on exclusive breast-feeding were comparable for both groups, indicating that the introduction of the iron-fortified formula had no effect on duration of breast-feeding. The percentage of infants born in August and September who actually consumed the acidified milk, excluding those who were breast-fed, varied between 70% and 80% from 3 to 15 months of age. Mothers who stated that their infants rejected the acidified milk were allowed to switch to the regular milk. It was difficult to determine whether it was the infant or the mother who rejected the acidified milk.

There was a highly significant difference ($p < 0.001$) in all laboratory parameters of iron status between the two groups measured at 9 and 15 months of age. The percentage of iron-deficient subjects was lower in the iron-fortified group. The incidence of anemia in the iron-fortified group was 11.8% at 8 months and 5.5% at 15 months of age, compared with 32.5% at 9 months and 29.9% at 15 months in the control group. At 15 months of age, only 3.8% of the infants who took iron-fortified milk for more than 10 months were anemic compared with 12.5% of those taking the iron-fortified milk for less than 10 months.

During the summer months of the southern hemisphere (November through February), when diarrhea tends to be prevalent, the group receiving iron-fortified milk had a lower incidence of diarrhea than the group receiving non-iron-fortified milk. The differences between the groups were statistically significant for the months of November and February. No group differences in the incidence of diarrhea were observed during the winter months. Moreover, there were no seasonal differences in the incidence of respiratory infections between the two groups.

In summary, the regional field trial confirmed the positive effect of the well-tolerated, acidified, iron-fortified milk on the iron nutritional status of

infants. There also appeared to be a positive effect of iron fortification on growth, particularly in low-birth-weight infants. This effect may have been due, in part, to less sharing of the acidified iron-fortified milk within the family. In addition, the acidified iron-fortified milk seemed to offer some protection against diarrhea in the summer months. However, the effect of the iron could not be separated from the effect of acidification.

Study 3. This study evolved from a recently completed field trial of iron-fortified foods.¹⁹ In this study, infants being breast-fed satisfactorily at 3 months of age were randomly assigned to one of two groups: either a group receiving heme-iron-fortified rice cereal as a weaning food at 4 months of age and continuing through 12 months of age, or a group receiving the common solid foods that caregivers of Chilean infants are instructed to use (a meat-cereal-vegetable soup, fruit, and fruit juices at 4 months, legumes at 6 months, and table foods at 9 months of age). Infants who were obtaining 50% of their anticipated energy intake from sources other than human milk were assigned either to a group receiving nonacidified fortified milk with 15 mg elemental iron as ferrous sulfate per liter of reconstituted milk plus 100 mg of ascorbic acid, or to a group receiving regular non-iron-fortified milk provided by the NHS. Each group consisted originally of approximately 100 infants.

All infants enrolled in the study were full term and essentially healthy. They were seen monthly at the clinic for checkup, anticipatory guidance, and anthropometry, and they could come to the clinic whenever they were ill. Each home was visited weekly by a field nurse who completed a dietary survey and morbidity questionnaire similar to that used in Study 1.

Growth and development were similar in all groups. Breast-fed infants tended to be heavier during the first 6 to 9 months of age, but their weights were at the 50th percentile of the U.S. National Center for Health Statistics Growth Curve (NCHS) by 1 year of age. Deficient iron status, measured by low iron stores and anemia, decreased in incidence across groups as follows: non-iron-fortified early weaned infants, unfortified breast-fed infants, iron fortified breast-fed infants, and fortified milk-fed infants.

These results illustrate the partial protection offered by breast-feeding and the effectiveness of feeding an iron-fortified product consumed consistently from 3 to 4 months age.

The incidence of mild diarrhea (duration of less than 1 day), diarrhea for more than 1 day, and upper respiratory or lower respiratory disease was identical in all groups. Otitis media was also uncommon.

Seasonal variation showed its usual influence on prevalence of diarrhea and respiratory disease without unduly affecting any particular set of infants.

In other studies from developed countries, Tunnessen and Osiki²⁰ reported on the effects of feeding whole cow's milk versus iron-fortified formula to infants after 6 months of age. At 12 months of age, the 69 infants receiving cow's milk had evidence of a lower iron status as measured by serum ferritin, erythrocyte protoporphyrin, and mean corpuscular volume, and an increased incidence of anemia, compared with 98 infants receiving iron-fortified formula. The incidence of otitis media, wheezing, nasal discharge or congestion, diaper rash, constipation, guaiac-positive stools, or hospital admissions did not differ. Diarrhea, however, was more frequent in the infants fed cow's milk. A bias in this study may be the parental decision to give cow's milk instead of an infant formula when both were provided free of charge.

In conclusion, where sanitation and disadvantaged living conditions considerably increase the susceptibility to infection, at least one study appeared to demonstrate that large doses of oral iron for adults and parenteral iron for 2-month-old infants moderately increased the incidence of certain illnesses, particularly malaria. However, in all of these subjects, because multiple nutritional deficiencies were not unequivocally distinguished, these effects could not be attributed exclusively to iron. Therefore, under usual circumstances in most areas of the world, oral iron therapy is not associated with increased rates of infection. More research is needed on the use of parenteral iron preparations in infancy, particularly in regions where there is a high prevalence of malaria. All current evidence indicates that iron fortification of foods is not associated with increased susceptibility to infection; moreover, there is some evidence that an adequate iron nutritional status may be beneficial.

Effect of Infection on Iron Metabolism and Anemia

Here, we consider the "anemia of chronic disease." Its pathogenesis is now thought to be due to immune activation from contact with either a foreign infectious agent or a foreign neoplasm. Immune activation releases cytokines, such as tumor necrosis factor, interleukin-1, gamma-interferon, and beta-interferon, which lead to the inhibition of colony-forming units—erythroid (CFU-E) development and produce anemia. This reduction in erythropoiesis can be overcome with pharmacologic doses of erythropoietin both in vivo and in vitro.

Pathogenesis and Treatment of the Anemia of Chronic Disease

The anemia of chronic disease (ACD) can be viewed simply as the anemia that accompanies chronic inflammatory, infectious, or neoplastic disorders. Because these conditions are very common, ACD is one of the most frequent anemias encountered and is only second in incidence to iron deficiency anemia. ACD is primarily due to underproduction of red cells, with low reticulocyte counts, and is most often a normochromic, normocytic anemia. However, in 30–50% of patients, the red cells are hypochromic and microcytic, and most often the serum iron, total iron-binding capacity, and transferrin saturation are reduced in the presence of adequate iron stores. Recently, major advances have occurred in our understanding of the pathogenesis of ACD and its treatment.

Serum Erythropoietin (EPO) Levels in ACD. A prime example of ACD is the anemia occurring with rheumatoid arthritis (RA). RA often serves as a model for ACD, and extensive investigations of serum EPO levels in ACD were performed in patients with RA.²¹ Although their serum EPO increased in response to anemia, the EPO levels attained were lower than those detected in equally anemic 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 slope of the line for the RA patients was shifted downward, indicating a blunted EPO response to anemia in RA patients.

Although 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 individuals without anemia. If normal people had the enhanced EPO levels of patients with ACD, they would be polycythemic. Thus, the failure of the bone marrow to respond to these increases in EPO must be considered the primary reason for the anemia.

Marrow Failure in ACD. Improved understanding of the pathogenesis of inflammation has come through the identification of cytokines that mediate this effect, their enhanced availability in highly purified or recombinant preparations, and the development of very sensitive immune assays for these proteins. This has allowed a more precise delineation of the mechanism by which erythropoiesis is inhibited in ACD.

As noted above, the anemia of RA often serves as a model for ACD. Levels of interleukin-1 (IL-1), a polypeptide that has a wide variety of actions in inflammation and immunity, are elevated in patients

with RA, as well as other ACD-associated conditions, and this elevation is directly proportional to the degree of anemia.²²

IL-1 has also been shown to inhibit murine CFU-E in vitro and in vivo. Murine burst-forming units—erythroid (BFU-E), which are more immature than CFU-E—as well as granulocytic, monocytic, and megakaryocytic progenitors, were stimulated by IL-1, with a maximum effect evident by 48 hours. Following repeated injections of IL-2, however, the mice became anemic. 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, whereas in vitro colony formation by marrow granulocyte-macrophage progenitors (CFU-GM) was not inhibited.

When the inhibition of human CFU-E colony formation by recombinant human IL-1 β (rhIL-1 β) was studied using both normal human bone marrow cells and highly purified human CFU-E, the inhibitory effect was indirect and required the presence of T lymphocytes. RhIL-1 β inhibited CFU-E colony formation by approximately 50% when added to human bone marrow cells in vitro, but did not inhibit it after the T cells were removed and no inhibitory effect of rhIL-1 β was observed on the highly purified CFU-E.²³ When autologous peripheral blood T lymphocytes were added to the highly purified CFU-E, rhIL-1 β significantly inhibited CFU-E, whereas the addition of cells that adhere to plastic Petri dishes (mostly monocytes) had little effect. A “conditioned medium” was obtained after the incubation of T lymphocytes with rhIL-1 β , and this medium also inhibited highly purified CFU-E colony formation, indicating that the T cells were producing a soluble substance that produced the inhibition. Antibodies to a variety of cytokines were added to the conditioned medium, and only antibody to gamma-interferon (IFN γ) neutralized the inhibitor of purified human CFU-E. To confirm that IFN γ inhibited the highly purified human CFU-E, recombinant human IFN γ (rhIFN γ) was incubated with these cells and a significant inhibition was observed with 10–1000 U/mL. Thus, rhIL-1 acted on T lymphocytes to produce IFN, and IFN in turn directly inhibited CFU-E colony formation.²³ This correlated well with previous studies implicating IFN in the pathogenesis of ACD. Because IFN inhibits colony formation by CFU-GM as well as by erythroid progenitors, this result would appear initially to be in conflict with any erythroid specificity. However, IL-1 leads to the release of granulocyte-macrophage (GM) and granulocyte (G) colony-stimulating factors (CSFs), which can overcome the inhibitory effects of IFN

on myeloid progenitors. CFU-E colony formation, which is not affected by G- or GM-CSF, would not be subject to “rescue” from inhibition by these growth factors.

Tumor necrosis factor alpha (TNF α) shares many of the biologic properties of IL-1 and, like IL-1, plays a significant role in inflammation and the immune response. TNF α levels have been reported to be increased in patients with cancer and RA, as well as parasitic, bacterial, and viral infections.²⁴

Chronic administration of TNF α to animals results in the development of anemia, and like ACD in humans, this anemia is associated with a low serum iron and normal iron stores. Patients given recombinant TNF α as experimental cancer therapy also developed anemia with a decline of hemoglobin of 24 g/L over 4 weeks, but the granulocyte and platelet counts remained unchanged. In vitro inhibition of human BFU-E and CFU-E formation by TNF α has also been demonstrated, and it was suggested that TNF α directly inhibited human erythroid progenitors. However, when highly purified human CFU-E was studied, it was found that the inhibitory effect of TNF α on CFU-E colony formation was indirect and required a soluble factor released from marrow stromal cells.²⁵ This factor was recently identified as beta-interferon.²⁶

Interferon gamma (IFN γ) is mainly produced by T lymphocytes and is involved in the modulation of immune and inflammatory responses and in the host defense against microbial challenge. Elevated IFN γ levels have been reported in patients with neoplastic and infectious diseases,^{27,28} and cancer patients treated with IFN γ developed a normochromic-normocytic anemia.²⁹ A number of investigators have reported inhibition of human erythroid colony formation in vitro by IFN γ . Mamus and coworkers,³⁰ studying both BFU-E and CFU-E colony formation, reported that the inhibitory effect of IFN γ was indirect and required accessory cells, whereas Raefsky et al.³¹ and Means et al.,²³ studying colony formation from purified progenitors, reported that this inhibitory effect was the result of a direct action of IFN γ on CFU-E.

Denz et al.²⁸ investigated the correlation between ACD and markers of immune activation such as IFN γ and neopterin. Neopterin is a pteridine that indicates activation of macrophages by IFN γ and is increased in a variety of infectious, inflammatory, and malignant disorders. These investigators studied 25 patients with hematological malignancies, 44% of whom were anemic (hemoglobin <120 g/L). Serum neopterin and IFN γ levels showed a significant inverse correlation with the hemoglobin, demonstrating a relationship between anemia and a mediator of

immune activation, as Eastgate et al.²² had previously shown for IL-1.

Many cytokines involved in the inflammatory and immune response, such as IFN α and transforming growth factor beta (TGF β), inhibit erythroid colony formation in vitro or are associated with the development of anemia. These might also merit investigation for a role in the pathogenesis of ACD. Moreover, in addition to their own effects, cytokines implicated in the pathogenesis of ACD can exhibit synergy. Synergistic inhibition of erythroid progenitors in vitro by IFN α and IFN γ has been reported, and IFN γ also shows synergy with TNF α in its inhibitory effect on hematopoiesis in vitro. In addition, various cytokines involved in the inflammatory response have amplification pathways. Both TNF α and IL-1 induce expression of the other cytokines and also increase their own expression.

Role of Erythropoietin (EPO) in ACD

EPO production is increased by tissue hypoxia, including anemia, and the serum EPO level is inversely correlated with hematocrit: as hematocrit falls, the serum EPO level rises, unless renal failure is present. Because of its primary importance in erythropoiesis, EPO has been a major focus of investigation in ACD.

Reduced EPO Production in ACD. Recent investigations have suggested that cytokines may be responsible for the reduced EPO response to anemia in ACD. Faquin et al.³² 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. Jelkmann and coworkers,³³ using the HepG2 line, reported similar results for IL-1 and TNF α but noted no inhibition by TGF β . In addition, they reported that IL-1 β inhibited EPO production by isolated perfused rat kidneys. By inhibiting EPO production as well as marrow erythropoiesis, cytokines such as IL-1 and TNF α may amplify their contributions to the development of ACD.

Response of ACD to EPO. The first patients with ACD to be treated with rhEPO were those who had RA.³⁴ Seventeen patients with RA were entered into a double-blind, randomized, placebo-controlled 8-week trial of rhEPO followed by a 24-week open maintenance phase. During the initial 8-week study, 13 patients received rhEPO and four received placebo. Four patients responded to rhEPO with an increase in the hematocrit of six percentage points or more: one of one who received 150 units/kg three times weekly intravenously, two of six who received 100 units/kg, and one of six who received 50 units/kg. None of the four patients who received placebo

had such a response.

When the patients were entered into the open maintenance phase, which permitted an increased time of treatment and an increased dose of rhEPO, eight of eight additional responses to rhEPO were evident in patients who had previously not responded. Thus, 12 of 16 RA patients who received rhEPO had a response, and 11 of 12 attained a normal hematocrit.

The RA patients received rhEPO intravenously, but because several studies have now shown that rhEPO is 25% more effective when administered subcutaneously, the latter is now the preferred route. This response to rhEPO might be useful for RA patients who are anemic and for RA patients who need surgery, since it is now possible for the latter to have surgery with a normal hematocrit or even to predonate blood with rhEPO treatment.

Because 50% of patients with acquired immunodeficiency syndrome (AIDS) who are receiving zidovudine therapy have anemia severe enough to require red cell transfusions, and because 70% of these patients have serum EPO levels less than 500 mU/mL, a double-blind placebo-controlled study of the effect of rhEPO on anemia was performed.³⁵ A total of 297 anemic (hematocrit <0.30) patients with AIDS were randomly assigned to receive either rhEPO 100–200 U/kg or placebo, intravenously or subcutaneously, three times per week for up to 12 weeks. In patients with EPO levels less than 500 mU/mL, rhEPO decreased the number of red cell transfusions per patient from 5.3 to 3.2 units and increased the mean hematocrit 0.046 compared with 0.005 in the placebo group. Patients with EPO levels greater than 500 mU/mL had no benefit from this treatment.

Ludwig et al.³⁶ treated 13 patients who had anemia secondary to multiple myeloma with 150 U/kg of rhEPO subcutaneously three times per week, with escalation of the dose by 50 units/kg every 3 weeks if there was no response. Response was defined as an increase in hemoglobin of at least 20 g/L. Two patients did not complete the study, but 11 patients responded to treatment with a median increase in hemoglobin of approximately 30 g/L plus a heightened sense of well-being and a significant increase in performance status.

In one of the largest cohorts of patients with anemia secondary to cancer, Abels³⁷ reported the treatment of 413 patients with an extremely wide variety of malignancies with 100–150 units/kg rhEPO subcutaneously three times per week for 8–12 weeks. Of those receiving chemotherapy, 40% had an incidence hematocrit correction to the target of 0.38, and the hematocrit of 58% of them increased

by at least 0.06 during this limited period, compared with randomized, placebo-treated patients, where the figures were only 0.04 and 0.14. Quality-of-life parameters also significantly improved in rhEPO-treated patients whose hematocrits increased by 0.06 or more compared with the placebo-treated patients.

Thus, rhEPO can correct ACD in most situations where it is encountered. It is of interest that serum IFN levels are increased in patients with AIDS and that an inverse relationship has been shown with the hemoglobin level.²⁷ Furthermore, the inhibition of highly purified human CFU-E colony formation by r γ IFN can be corrected by exposure to very high concentrations of rhEPO in vitro,³⁸ just as ACD is corrected by rhEPO in these patients. Since the development of the CFU-E plasma clot method to the present time, it has been repeatedly demonstrated that 1 U/mL of EPO is the maximum effective dose of the hormone in vitro because this dose saturates most of the EPO receptors. However, in the presence of an IFN-induced inhibition of CFU-E in vitro, the amount of rhEPO required to overcome the inhibitory effect depends on the amount of IFN, and ranges from 16 U/mL rhEPO in the presence of 100 U/mL rhIFN to 64 U/mL rhEPO in the presence of 1000 U/mL rhIFN.³⁸ Thus, the in vivo correction of ACD by pharmacologic concentrations of rhEPO can be replicated in vitro, and this makes possible further study of the mechanism of this anemia.

Altered Iron Metabolism in ACD

ACD is often associated with low serum iron in the presence of adequate reticuloendothelial (RE) iron stores, and this has led to extensive investigations of iron metabolism. Early investigations demonstrated a block in the release of RE iron and implied that ACD involved a functional iron deficiency that inhibited erythropoiesis. However, other studies showed increased ineffective erythropoiesis that appeared not to be related to functional iron deficiency. Recent studies of the role of cytokines confirm that both impaired iron metabolism and impaired erythropoiesis are involved in ACD. A direct correlation between the degree of anemia and levels of serum neopterin (a marker of immune activation associated with anemia in ACD patients), IFN, and ferritin was reported in patients with HIV infection, suggesting a role for immune activation in altered iron metabolism.²⁷ Low hemoglobin was associated with low transferrin and low free-iron-binding capacity.

In ACD, impairment of iron metabolism and erythropoiesis is present, but several reasons exist for believing that the latter is more important. First, rhEPO can correct ACD but cannot correct the ane-

mia of iron deficiency. Second, iron ingestion, which increases serum ferritin levels, has no effect on the anemia of RA, but EPO normalizes the hematocrits of RA patients. Third, serum transferrin receptor levels are elevated in patients with iron deficiency but are generally not elevated in patients with ACD. Because EPO acts on erythroid cells and not RE cells, it is likely that the immune cytokines are decreasing erythropoiesis and altering iron metabolism within the erythroid cells as the prime event and that any effect on the RE cells is a secondary manifestation.

Effect of Acute Infections in Infancy on Iron Status Measures

Chronic inflammatory disease and severe bacterial processes are well-recognized causes of anemia.^{39,40,41} This reduction in hemoglobin levels is in part due to a block in iron release from the RE system, with the consequent reduction of iron available for erythropoiesis. Both a reduction of serum iron and an increase in serum ferritin are a common finding.^{39,40}

These changes are well characterized in adult patients with such diseases as rheumatoid arthritis. This model is inadequate in childhood, where the majority of mild to moderate febrile illnesses are acute and of viral origin. There is less information on hematologic changes in these conditions. In two well-executed retrospective studies, children who had a history of mild infections in the previous 1 to 3 months had a higher incidence of anemia, particularly infants younger than 12 months.^{42,43} The association of anemia with a high sedimentation rate was also noted. To further define these changes, we studied 15 children between the ages of 6 months and 10 years who were seen for common acute febrile infections in an outpatient clinic. A venous blood sample was obtained at diagnosis and after 30 days. The latter was considered the baseline. During the acute infection there was a statistically relevant fall in hemoglobin, serum iron, and transferrin saturation and a statistically significant increase in serum ferritin.⁴⁴

In childhood there are also many mild, short-lived infections that would usually not warrant medical consultation. In a prospective study, we used immunization with attenuated measles virus as a model of a predictable mild viral illness.^{45,46} We observed a significant drop in hemoglobin concentration by days 9 to 14 postvaccination. The decrease was >10 g/L in 8.6% of the infants. More relevantly, anemia was overdiagnosed in 22% of the infants who were not anemic before immunization. Serum iron and transferrin saturation fell significantly, whereas erythrocyte protoporphyrin and serum ferritin increased significantly. Most of the changes in iron

parameters persisted for 2 or 3 weeks after the appearance of fever, and some measures may have even become abnormal during the incubation period of viral illness. These modifications of the laboratory measures of iron status were more prominent in subjects with increased C reactive protein, high band counts, or fever above 38 °C.^{47,48}

Proper diagnosis of iron status is based primarily on laboratory measures. However, in populations where infections are prevalent, classic iron measures might underestimate or overestimate the prevalence of iron deficiency depending on the measure used. Reliance on biochemical measures, or in some cases hemoglobin, may be inappropriate up to several weeks after a mild infection. The recent appearance of a new assay, the serum transferrin receptor, has been a useful addition to the clarification of this pitfall. This parameter is sensitive to detect mild iron deficiency⁴⁹ and is not affected by acute or chronic infections.^{50,51}

Effect of Iron Deficiency Anemia on Immune Function In Vitro and the Production of Cytokines

Immune Response In Vitro

Two immune system abnormalities associated with iron deficiency have been documented in humans: an impaired response of T lymphocytes to mitogens and a decreased bactericidal activity of neutrophils. The DNA synthesis of T lymphocytes in response to stimulants or mitogens results in “blastic transformation” and the production of lymphokines that are important for immune regulation. A continuous supply of iron is required for the activity of mammalian ribonucleotide reductase,⁵² an obligatory step in DNA synthesis.⁵³

Joynson et al.⁵⁴ described an impairment in lymphocyte transformation and production of migration inhibition factor after *Candida* and purified protein derivative (PPD) antigen stimulation in 12 subjects with iron deficiency anemia. Both the proportion and the absolute number of T lymphocytes were reduced in iron deficiency anemia. Lymphocyte proliferation and response to phytohemagglutinin (PHA) and Concanavalin A (ConA) antigens were impaired in iron deficiency without anemia, and there was a significant correlation between the stimulation index and transferrin saturation.^{55,56}

In a recent study of 10 iron-deficient children 12 to 30 months old, the mean stimulation index for *Candida* antigen increased from 6.8% to 17.9%, and for tetanus antigen from 19.5% to 31.7%, following iron therapy.⁵⁷

A defect in neutrophil function in iron-deficient

patients could also predispose them to bacterial infection. Although it is generally agreed that phagocytosis, or ingestion of bacteria, is normal in the presence of iron deficiency,^{58,59} the capacity for killing certain types of bacteria once they have been ingested is impaired.⁶⁰⁻⁶³

At least two or three iron-dependent steps are involved in intracellular bacteria killing. A sharp increase in oxygen consumption or the “respiratory burst”⁷⁶⁴ results from the activation of NADPH oxidase (presumably an iron-sulfur enzyme), which produces O₂ and H₂O₂. The heme protein cytochrome b is also associated with the “respiratory burst” in a way yet to be clarified. H₂O₂ and O₂ are used to produce oxidized halogens and OH⁻ radicals, which are effective in bacterial killing. The heme iron enzyme myeloperoxidase mediates the halogenation of bacterial protein using H₂O₂. The production of OH⁻ radicals is catalyzed by the iron present in leukocyte lactoferrin by way of the Haber-Weiss reaction.⁶⁵

Walter et al.⁶⁶ studied neutrophil function in 10 iron-deficient but otherwise healthy infants 6 to 23 months of age. Neutrophil function and iron status were assessed at 0, 3-5, 15, 30, and 90 days after oral iron therapy had been initiated. Although phagocytosis was unaffected, bactericidal activity was profoundly impaired before therapy, improved partially at 3 to 5 days, and was completely corrected at 15 days (Figure 1). The timing of recovery suggested that iron had no effect on circulating neutrophils but was required for neutrophil development in the bone marrow. This finding is in accordance with the rate of recovery of myeloperoxidase activity in iron-deficient rats. In these iron-deficient rats, however, the “oxidative burst” was maintained, allowing other bacterial killing mechanisms to continue.⁶⁷ This finding in the animal model may explain why no overt clinical signs (respiratory, gastrointestinal, or cutaneous) could be identified in this group of children either before they were studied or during the subsequent 15 days of close clinical and laboratory follow-up, in spite of profound in vitro immune defects. The clinical significance of these laboratory findings of immune function defects in moderately to severely iron-deficient infants is questionable.

The preceding studies provide convincing evidence of an unfavorable effect of iron deficiency on human T-cell and phagocyte function in vitro. In addition, bacteria require iron for growth, and increased iron availability enhances bacterial virulence. In fact, iron is avidly bound by bacterial iron transport cofactors called siderophores. The iron-binding affinity of siderophores is comparable to that of transferrin. Several in vitro experiments have shown

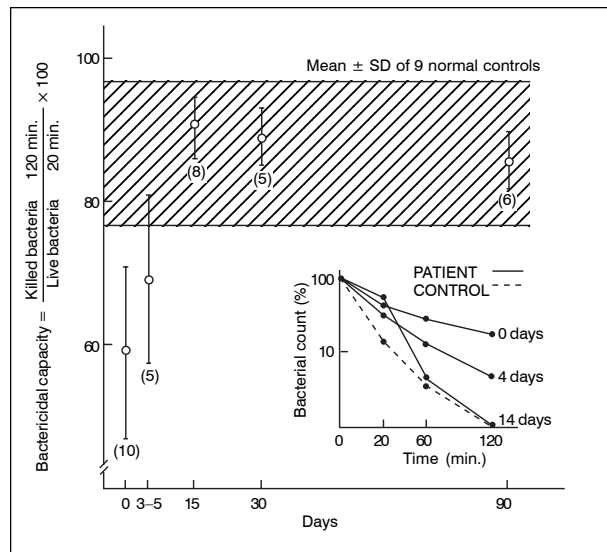


Figure 1. Effect of therapy on the bactericidal capacity of iron-deficient infants. Bactericidal capacity is expressed as the percent of killed bacteria at 120 minutes over live bacteria at 20 minutes. Values are expressed as mean \pm SEM. The hatched area represents normal control range. The inset in the lower right corner represents a typical assay of one patient.

that the addition of unsaturated transferrin or iron chelators such as desferrioxamine to culture media inhibits bacterial growth and that bacteria resume growth with iron replacement.⁶⁸

Less iron is available to bacteria during an infectious process owing to the “iron shift” that occurs as part of the acute-phase reaction. The iron shift involves a rapid decrease in serum iron concentration with a consequent fall in transferrin saturation (reviewed above). Unsaturated transferrin could compete for available iron sources and contribute to an inhibition of microbial growth and a decrease in virulence.

Iron Deficiency Anemia and Cytokine Production

Iron deficiency anemia infection alters the complex balance between the mineral requirements of the host and the invading microorganism, and the role of the iron in mounting an effective immune response in the host. Iron deficiency is known to affect preferentially cell-mediated immune functions including delayed hypersensitivity skin response, lymphocyte proliferation to mitogens and natural killer cytotoxicity, and so on.⁶⁹⁻⁷¹ Most of these anomalies revert to normal after iron therapy.⁷⁰ However, sometimes confounding variables in human studies such as inadvertent additional nutritional deficiencies or pre-existing infection make it hard to conclude that iron is the only culpable factor in the immune alterations.

Precisely how iron and its deficiency alter immunity is not fully understood. Multifactorial mecha-

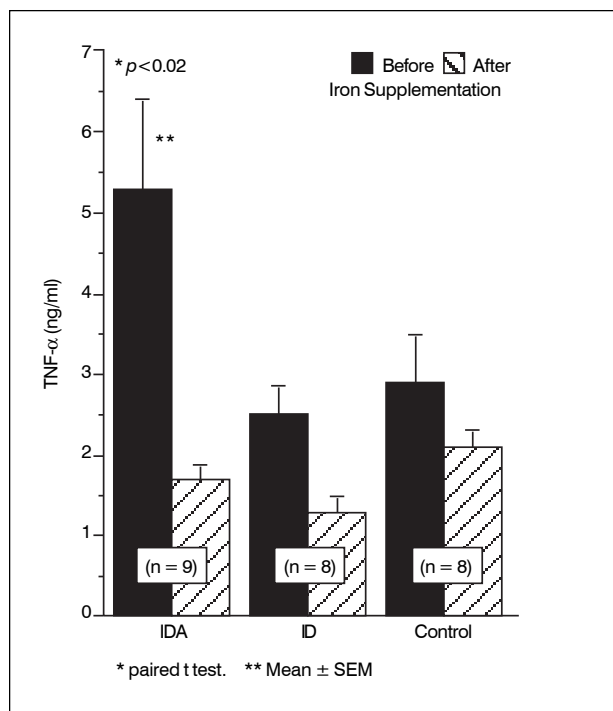


Figure 2. Effect of iron supplementation on in vitro TNF α production in iron deficiency with anemia (IDA), in iron deficiency without anemia (ID), and in controls. Values expressed are mean \pm SEM.

nisms could be responsible for impaired host defenses such as alteration in the activity of the iron-dependent enzyme protein kinase C,⁷² synthesis of transferrin receptors,⁷³ and so on. A few recent studies in animals and humans have also postulated modifications in the production of intracellular messengers including interleukins 1 and 2 (IL-1, IL-2).⁷⁴⁻⁷⁹ Among monocyte-derived cytokines, the polypeptide TNF α plays a key role in hematopoiesis and iron metabolism.⁸⁰⁻⁸² We recently examined⁸¹ in vitro production of this cytokine in blood mononuclear cells (BMNC) from subjects with iron deficiency anemia (IDA), iron deficiency without anemia (ID), and control infants before and after 3 months of iron supplementation (3 mg ferrous sulfate drops/kg/day). Lipopolysaccharide-stimulated blood monocytes from IDA infants ($n=9$) produced a significantly higher immunoreactive TNF α concentration compared with ID ($n=9$) and normal subjects ($n=18$) on admission ($F=6.72$, $p<0.004$). After iron therapy, TNF production by cells from IDA infants returned to the levels observed in the other groups (Figure 2). The increased TNF α production by BMNC in IDA subjects could intensify the decreased erythroid proliferation observed in this condition. This response may be induced by a block to iron release from the reticuloendothelial system and/or by inhi-

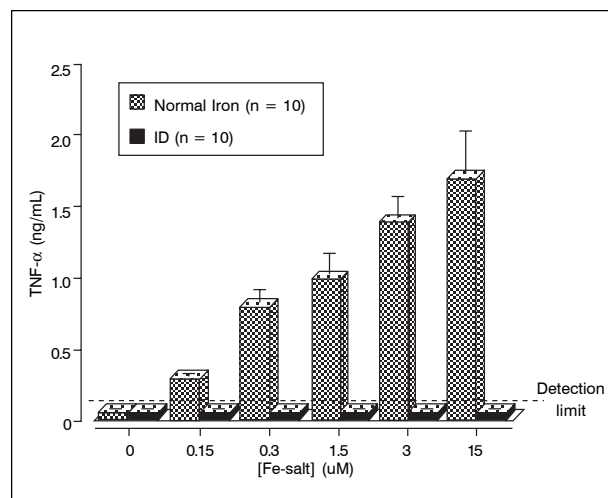


Figure 3. Effect of the addition of iron-salt on TNF α secretion by BMNC from normal iron and ID subjects. Values shown as mean \pm SEM. Detection limit of technique < 50 pg/mL. BMNC = blood mononuclear cells. ID = iron deficiency.

bition of erythropoiesis.

To investigate whether iron modulates TNF α secretion by human monocytes, we studied the effects of in vitro addition of the iron salt ferric chloride (FeCl₃) to monocytes derived from subjects with IDA and compared them with age- and sex-matched normal-iron-status individuals. Well-nourished adult women (25–54 years old) free of acute infections at least 15 days before admission were included in this study. The effect of FeCl₃ on TNF α secretion was tested by incubating cells from both groups of subjects with increasing concentrations of FeCl₃ (range 0–15 μ M). The addition of iron to cells from controls stimulated TNF α secretion in a concentration-dependent manner, suggesting that Fe⁺³ is a potent inducer of this cytokine (Figure 3).

Similar findings were recently reported after the addition of copper and zinc salts and ferric ammonium citrate (FAC) to peripheral blood leukocytes derived from healthy donors with adequate mineral status.^{82,83} The mechanism by which iron stimulates TNF α secretion in normal cells is at present unknown. The requirement of this metal as a component of many metalloenzymes and the demonstration that in vitro secretion is dependent on protease activity⁸⁴ suggest that the trace element may be involved in some essential catalytic role as a rate-limiting factor in TNF α production and secretion. For instance, the iron-dependent enzyme protein kinase C (PKC) is one of the protein kinases that regulate TNF α secretion at a transcriptional level.⁸⁵ When BMNC from both anemic and normal donors were cultured alone in complete medium without exogenous ferric salts, cells consistently did not secrete TNF α (levels under the detection limit of the spe-

cific ELISA technique [< 50 pg/mL]). This evidence suggests that the observed enhancement with increasing doses of FeCl_3 in normal subjects was not due to contaminating endotoxin.

By contrast, in our study, cells from women with IDA did not secrete $\text{TNF}\alpha$ after incubation with different concentrations of iron salts. We hypothesize that the absence of response in anemic subjects could be due to depressed activity of the iron-dependent PKC and/or alteration in the transferrin-independent iron transport. Studies in spleen cells from female mice fed iron-deficient diets show a decline of PKC activity and poor iron translocation resulting in aberrant signal transduction, which in turn might be responsible for the impaired lymphocyte proliferation associated with iron deficiency.⁷² However, several recent reports have characterized the importance of the iron independence of the transferrin-mediated pathway by a variety of cell systems⁸⁶⁻⁸⁸ where regulation is independent of cellular iron requirements and growth states. Kaplan et al.⁸⁹ demonstrated that the activity of this transport system is increased when HeLa cells are exposed to high doses of ferric salts such as FeNH_4 citrate ($1-10$ μg Fe/mL). These concentrations were similar to those used in our study.

Understanding the role of iron in the secretion and functions of cytokines in pathologic conditions could help design dietary modifications for prevention and therapy of diseases associated with iron deficiency.

Conclusion

Existing research yields conflicting results regarding the relationship between iron and infection. Several confounding factors cloud the data surrounding whether or not iron treatment increases the risk for infection. Further research is necessary to determine the effects of iron deficiency anemia on immune function and the effects of infection on iron status.

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