A Review on Iron Homeostasis and Anaemia in Pulmonary Tuberculosis

Obeagu Emmanuel Ifeanyi

Diagnostic Laboratory unit, Department of University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract

The paper discussed iron homeostasis and anaemia in pulmonary tuberculosis. Iron (Fe) is one of the most abundant elements in the earth's crust and an essential nutrient for almost all known organisms. It is able to receive and release electrons during conversion from Fe (II) to Fe (III) and plays a major role in DNA production and in energy generation. A system of highly regulated mechanisms is in place to control iron homeostasis. Regulation occurs at both the systemic and cellular levels and influences a number of iron-associated proteins. The iron regulatory protein, hepcidin, plays an important role in the mechanisms responsible for AI and the inflammation-mediated alteration of iron homeostasis. Specifically, hepcidin binds to and degrades the iron export protein ferroportin and down-regulates expression of the iron importer DMT1. Since hepcidin transcription is induced by the pro-inflammatory cytokine, interleukin-6 (IL-6), inflammation leads to a reduction in iron absorption and causes iron to be sequestered in macrophages and enterocytes. The inflammatory cytokines that are released upon innate recognition of a pathogen induce changes in plasma concentrations of other proteins. This phenomenon is referred to as the acute phase response (APR) and the affected proteins are considered acute phase proteins (APPs). The iron homeostasis proteins ferritin, transferrin and hepcidin are considered APPs, as are C-reactive protein (CRP) and alpha-1-antichymotrypsin (ACT), both of which are often utilized in studies as markers of inflammation. In the context of infection, iron's limited availability within the human body and its physiological importance to both hosts and microbes make it a valuable commodity. Many microbes depend on host-acquired iron and, in response; hosts use their complex system of iron regulation to modify their iron metabolism and restrict iron availability. Evidence links iron with PTB pathogenesis both from the perspective of the pathogen and the host. Upon infection, host immune recognition of Mtb induces a pro-inflammatory reaction that restricts iron access. Most strikingly, a pattern of altered host iron status characterized by high ferritin, low transferrin, and low hemoglobin has been identified as a risk factor for progression to PTB. Decreasing iron availability (regardless of the mechanism) reduces Mtb growth, and addition of iron to Mtb almost always enhances growth.

Keywords: Iron homeostasis; Anaemia; Pulmonary tuberculosis.

1. Introduction

Iron (Fe) is one of the most abundant elements in the earth's crust and an essential nutrient for almost all known organisms. It is able to receive and release electrons during conversion from Fe (II) to Fe (III) and plays a major role in DNA production and in energy generation [1]. In humans, iron is considered indispensible because it serves as a co-factor for many proteins, including hemoproteins and enzymes, which are essential for fundamental cellular processes [2].

At a given time, between three and five grams of iron are present in the human body, with the majority in the form of haeme in hemoglobin. Macrophages, muscle myoglobin, and the liver parenchyma also contain significant portions of body iron. Absorption of dietary inorganic iron occurs through enterocytes via divalent metal transporter 1 (DMT1) following reduction of Fe (III) to Fe (II). Internalization of dietary haeme also occurs through enterocytes. Export of iron from enterocytes is mediated by ferroportin and coupled with re-oxidation from Fe (II) to Fe (III) by either the ferroxidase hephaestin or its homologue, ceruloplasmin. Once exported, iron binds to the iron transport protein, transferrin, for delivery to tissues and erythroblasts.

The potential consequences of "too much" or "too little" iron in the human body are dire. They include oxidative damage, anemia and increased susceptibility to infection. To avoid these consequences, a system of highly regulated mechanisms is in place to control iron homeostasis. Regulation occurs at both the systemic and cellular levels and influences a number of iron-associated proteins including the five proteins: ferritin, hepcidin, transferrin, soluble transferrin receptor (sTfR) and hemoglobin [2].

2. Anaemia

When hemoglobin concentration falls below a certain cutoff (which varies by age, sex and race [3], oxygen delivery to tissues is considered to be impaired and the individual with low hemoglobin is considered to be anaemic. Anaemia affects an estimated one-quarter of the world's population [4], with a disproportionate share of cases occurring in sub-Saharan Africa [5]. Consequences of anemia include increased morbidity and mortality [6], decreased quality of life [6], and diminished productivity [6]. Causes of anemia are diverse and often act in combination with each other. While the largest worldwide contributor to anemia is thought to be iron-deficiency [3],

factors including hemoglobinopathies, other micronutrient deficiencies and infection also play important roles in contributing to the worldwide anemia burden [4].

While determinants of iron deficiency anemia (IDA) are diverse (e.g., parasitic worms, diet, and pregnancy), the mechanisms responsible for IDA are straightforward: either decreased iron supply impairs hemoglobin production, or bleeding causes the loss of erythrocytes faster than they can be replaced. Anemia of inflammation (AI) (also referred to as "anemia of chronic disease (ACD)"), is considered to be the second largest contributor to anemia after iron deficiency [7]. It is a particularly important factor to consider in settings where anemia co-exists with high rates of infection. AI arises as a result of an innate immune response, in which pattern-recognition receptors on monocytes, macrophages, neutrophils and dendritic cells recognize motifs specific to pathogens and release inflammatory cytokines. The resulting inflammation leads to general alteration of iron homeostasis as well as anemia-causing pathologies including short erythrocyte life-span, poor erythrocyte iron incorporation and decreased sensitivity to or supply of erythropoietin [8].

The iron regulatory protein, hepcidin, plays an important role in the mechanisms responsible for AI and the inflammation-mediated alteration of iron homeostasis. Specifically, hepcidin binds to and degrades the iron export protein ferroportin and down-regulates expression of the iron importer DMT1.Since hepcidin transcription is induced by the pro-inflammatory cytokine, interleukin-6 (IL-6), inflammation leads to a reduction in iron absorption and causes iron to be sequestered in macrophages and enterocytes.This mechanism [9], contributes to the overall alteration of ironhomeostasis that is a hallmark of AI and also limits iron delivery to erythroblasts, functionally causing anemia [10, 11].

3. Acute Phase Response

In addition to lower hemoglobin concentrations, the inflammatory cytokines that are released upon innate recognition of a pathogen induce changes in plasma concentrations of other proteins. This phenomenon is referred to as the acute phase response (APR) and the affected proteins are considered acute phase proteins (APPs). The iron homeostasis proteins ferritin, transferrin and hepcidin are considered APPs, as are C-reactive protein (CRP) and alpha-1-antichymotrypsin (ACT), both of which are often utilized in studies as markers of inflammation. Changes in APPs must be considered when interpreting concentrations of iron homeostasis proteins. For example, a study in rural Zambian children reported that measurements of serum ferritin were 279-356% higher for those experiencing infection [12].

4. Iron and Infection

In the context of infection, iron's limited availability within the human body and its physiological importance to both hosts and microbes make it a valuable commodity. Many microbes depend on host-acquired iron and, in response; hosts use their complex system of iron regulation to modify their iron metabolism and restrict iron availability. Microbes, in turn, employ complex iron acquisition strategies to obtain the restricted resource [13].

Since microbes cultured in iron-scarce environments show decreased energy and nucleic acid production the nutritional immunity suggests that iron-deficient host environments are better equipped to resist infection than iron-rich host environments. Iron deficiency or 'too little iron' is known to compromise cell-mediated immune function [14] and iron sufficiency or 'too much iron' increases iron availability to pathogens leading to increased disease susceptibility [14-16]. The optimal or 'just right' level of iron status, which has not been defined, may maintain immune function while also conferring some protection against pathogens.

5. Pulmonary Tuberculosis Prevalence

Estimates suggest that approximately one-third of the world's population is infected with PTB causing bacteria, also known as the *Mycobacterium tuberculosis* (*Mtb*) Complex (MTBC) [7].

While the vast majority of infected individuals will never progress from latent PTB infection (LTBI) to active PTB disease, PTB is widespread and considered the world's second leading cause of infectious disease mortality. World Health Organization estimates for 2013 indicated that there were 9.0 million new cases of PTB and 1.5 million deaths from PTB worldwide, with the bulk of both cases and deaths occurring in Africa and Southeast Asia [17].

6. Pulmonary Tuberculosis Prevalence Pathogenesis

While there are several mycobacterial species that make up the MTBC and cause PTB, most cases of active PTB disease are attributed to *Mycobacterium tuberculosis (Mtb).* Transmission of *Mtb* occurs when an individual with active pulmonary TB disease coughs, sneezes, or shouts – liberating tiny airborne particles of tubercle bacilli that can be inhaled by anyone in close proximity. Inhaled bacilli make their way to the alveoli of the lungs where they are phagocytosed (internalized) by alveolar macrophages. Phagocytosis is followed by maturation of the phagosome, a process in which the chamber containing the *Mtb* bacilli is subject to acidification, production of reactive oxygen/nitrogen species, and release of anti-microbial peptides. Phagosomal maturation is often sufficient to destroy pathogens, but *Mtb's* unique cell wall enables it to tolerate and in some cases impair the phagosomal maturation process [18, 19].

Infected macrophages move from the airways into pulmonary tissue where a local inflammatory response initiates formation of a granuloma. The granuloma is a hallmark of PTB and is characterized by a core of infected macrophages surrounded by monocytes and T lymphocytes. In most people infected with *Mtb*, granuloma formation

signals that the host immune system has mostly managed to halt the replication and spread of *Mtb*. This stage of pathogenesis is known clinically as latent PTB infection (LTBI).During LTBI, the host immune [8] system keeps *Mtb* in check through deployment of phagosomal defense mechanisms and promotion of unfavorable conditions within the granumloma, while *Mtb* enters a state of dormancy in which its metabolic activity decreases and its ability to resist host defense mechanisms increases [20]. LTBI is asymptomatic and may be short lived or last for the individual's remaining years of life.

While some individuals progress directly from *Mtb* infection to active TB disease, most experience some form of LTBI between infection and active TB disease [21]. In the 5-10% [21] of individuals who progress from LTBI to active PTB disease, dormant *Mtb* reactivates and begins replicating within the granuloma. Due in part to increased metabolic activity by *Mtb,* the granuloma's previously solid center becomes caseous and begins to lose its structure. Eventually the unstructured granuloma ruptures, releasing TB bacilli into the airways [20].Some studies have speculated that resuscitation-promoting factor (Rpf) plays a role in inducing reactivation [22].The *Mtb* genome contains several Rpf orthologs [22] and a 2010 study showed that addition of Rpf to sputum from PTB patients increased recovery of *Mtb [23]*. Another model suggests that 'scout' *Mtb* organisms may determine whether or not broader *Mtb* reactivation will occur by sensing the attractiveness of the environment for replication [20]. In this model limitation of nutrients, including iron, would likely play an important role in determining the likelihood of *Mtb* reactivation.

7. Pulmonary Tuberculosis Prevalence Diagnosis and Treatment

Sustained high levels of new PTB cases and deaths from PTB serve as evidence that diagnosis and treatment of PTB present major challenges. PTB diagnosis has traditionally been [9] based on a "passive case finding" strategy, in which symptomatic individuals who presented at medical facilities were tested for the disease using sputum-smear microscopy and mycobacterial culture. While passive case finding, microscopy and culture are still in wide use, recent advances have improved case identification and accelerated PTB diagnosis. In particular, community based case finding strategies (e.g., enhanced case finding, in which communities are sensitized about PTB signs/symptoms and PTB contacts are screened) and the Xpert MTB/RIF test have had recent impacts [24, 25]. These developments are proving to be especially important in resource restricted settings where PTB prevalence is high and access to laboratories outfitted for PTB culture is decreased.

Upon diagnosis, current treatment guidelines for active TB disease include an intensive phase of the anti-TB drugs isoniazid, rifampicin, ethambutol, and pyrazinamide for two months, followed by isoniazid and rifampicin for four months [26]. Isoniazid and ethambutol work against PTB causing bacteria by interfering with mycobacterial cell wall production, rifampicin inhibits bacterial RNA synthesis and pyrazinamide impairs mycobacterial fatty acid synthesis. With timely diagnosis these drugs are extremely effective. Cure rates for patients (non-multi-drug resistant TB) on PTB treatment are close to 90% [27]. However, cure rates for untreated PTB cases or for patients in whom treatment is delayed, are poor. 70% of adults with untreated PTB die within 10 years [28] and delayed PTB treatment is linked to increased risk of mortality [29]. In addition, foregoing or delaying treatment fundamentally increases the likelihood of transmission of PTB causing bacteria [30]. Similar to patients who experience delays in PTB treatment, PTB patients with co-morbidities who are undergoing treatment are at higher-risk for poor PTB outcomes. Anemia at PTB diagnosis, for example, has been linked to an increased risk of Prentice*, et al.* [10] death [31, 32]. The same is true for individuals with malnutrition , bacterial pneumonia (Shimazaki *et al.,*2013), and HIV/TB co-infection [33]. Thus, timely diagnosis, swift administration of treatment, and resolution of co-morbidities are key components in reducing PTB transmission and death from PTB.

8. LTBI Screening and Treatment

The standard screening method for LTBI is the mantoux tuberculin skin test (TST). The TST is administered as an intradermal injection of a standard volume of tuberculin purified protein derivative (PPD) into the inner-forearm. Results of the test are based on the diameter of the induration 48 to 72 hours following administration. While the TST is generally effective, results require several days to obtain and specificity is low in populations vaccinated with the TB-vaccine, BCG, due to overlapping antigens present in both the vaccine and PPD [34]. These issues likely spurred the development of interferon gamma release assays (IGRA), which yield results within 24 hours and utilize antigens not found in BCG [35].

Treatment of individuals at risk for transitioning from LTBI to active PTB disease is an important strategy in fighting the global PTB epidemic. Treatment for LTBI typically includes some combination of self-administered isoniazid and/or rifampetine and is considered to be highly effective in decreasing the probability of progressing to active PTB disease [36]. The challenge in implementing treatment of LTBI is identification of when and in whom treatment should be initiated. The global population with LTBI includes an estimated two billion people, and it is unlikely that all of these individuals could or should be subjected to a lengthy course of antibiotics. Thus, an important step in treating LTBI will be identifying those individuals who are at risk of progressing to PTB and in need of treatment. The ability to identify who is at risk for [11] transitioning from an infected and stable state to an infected but disease progressing state is critical for both the clinical management of the individual and for the prevention of PTB transmission at the population level.

9. Role of Iron in Pulmonary tuberculosis

Evidence links iron with PTB pathogenesis both from the perspective of the pathogen and the host. Upon infection, host immune recognition of *Mtb* induces a pro-inflammatory reaction that restricts iron access [37].Since host-acquired iron is a co-factor involved in vital *Mtb* cellular processes, *Mtb* responds by manufacturing siderophores, molecules capable of binding iron more strongly than host iron-storage proteins. Specifically, *Mtb* synthesizes two types of siderophores: lipophilic, cell-bound mycobactins and free carboxymycobactins, which work together to capture iron from a variety of host iron sources (including transferrin, lactoferrin and haeme [38, 39] and transfer it across the *Mtb* cell wall. This process has been shown to be essential for *Mtb* growth and virulence [40, 41] and capable of increasing iron availability in the mycobacterial phagosome almost 20-fold [42]. Evidence highlighting the importance of siderophores to *Mtb* growth is consistent with studies examining the effect of modified iron status on *Mtb* growth. Decreasing iron availability (regardless of the mechanism) reduces *Mtb* growth, and addition of iron to *Mtb* almost always enhances growth [37].Similarly, genetics support the important role for iron in *Mtb* growth. *Mtb* mutants with defective genes involved in siderophore synthesis [40] and/or iron-utilization machinery show reduced growth [43].

Examination of the role of iron in PTB from the perspective of the host echoes its importance. Most strikingly, a pattern of altered host iron status characterized by high ferritin, low transferrin, and low hemoglobin has been identified as a risk factor for progression to PTB [44]. This is consistent with evidence indicating that high macrophage iron stores are linked to an increased likelihood of contracting *Mycobacterium spp.* infections as well as studies suggesting that dietary iron overload is associated with an increased risk of developing pulmonary tuberculosis (PTB) [45] and dying from PTB . It is also consistent with studies that have identified anemia is a risk factor for poorer PTB outcomes. From the genetic perspective, polymorphisms in the phagolysosomal iron transporter *SLC11A1* (NRAMP1) are associated with TB susceptibility [46, 47] and hereditary hemochromatosis, which leads to iron deficient macrophages, is thought to be protective against TB [48].

10. Conclusion

Iron (Fe) is one of the most abundant elements in the earth's crust and an essential nutrient for almost all known organisms. It is able to receive and release electrons during conversion from Fe (II) to Fe (III) and plays a major role in DNA production and in energy generation. A system of highly regulated mechanisms is in place to control iron homeostasis. Regulation occurs at both the systemic and cellular levels and influences a number of iron-associated proteins.

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