

Cigarette Smoke Particle-Induced Lung Injury and Iron Homeostasis

Andrew J Ghio¹
Elizabeth N Pavlisko²
Victor L Roggli²
Nevins W Todd³
Rahul G Sangani⁴

¹Human Studies Facility, US Environmental Protection Agency, Chapel Hill, NC, 27514, USA; ²Department of Pathology, Duke University, Durham, NC, USA; ³Department of Medicine, University of Maryland, Baltimore, MD, 21201, USA; ⁴Department of Medicine, West Virginia University, Morgantown, WV, USA

Abstract: It is proposed that the mechanistic basis for non-neoplastic lung injury with cigarette smoking is a disruption of iron homeostasis in cells after exposure to cigarette smoke particle (CSP). Following the complexation and sequestration of intracellular iron by CSP, the host response (eg, inflammation, mucus production, and fibrosis) attempts to reverse a functional metal deficiency. Clinical manifestations of this response can present as respiratory bronchiolitis, desquamative interstitial pneumonitis, pulmonary Langerhans' cell histiocytosis, asthma, pulmonary hypertension, chronic bronchitis, and pulmonary fibrosis. If the response is unsuccessful, the functional deficiency of iron progresses to irreversible cell death evident in emphysema and bronchiectasis. The subsequent clinical and pathological presentation is a continuum of lung injuries, which overlap and coexist with one another. Designating these non-neoplastic lung injuries after smoking as distinct disease processes fails to recognize shared relationships to each other and ultimately to CSP, as well as the common mechanistic pathway (ie, disruption of iron homeostasis).

Keywords: iron, ferritins, pulmonary disease, chronic obstructive, pulmonary emphysema, chronic bronchitis, hypertension, pulmonary, pulmonary fibrosis

Introduction

Cigarette smoking is associated with non-neoplastic lung injuries including bronchiolitis/pneumonitis, asthma, pulmonary hypertension, chronic bronchitis, pulmonary fibrosis, emphysema, bronchiectasis, and chronic obstructive pulmonary disease (COPD). The mechanistic pathway by which cigarette smoking initiates these injuries remains to be defined. It is proposed that the basis for non-neoplastic lung injuries associated with smoking is disruption of iron homeostasis in cells after exposure to cigarette smoke particle (CSP) (Figure 1). Following the complexation of intracellular iron by the CSP, inflammation, mucus production, and fibrosis attempt to reverse a functional metal deficiency. If the deficiency in cell iron is not corrected, the response progresses to include injuries associated with irreversible cell death including emphysema and bronchiectasis.

Clinical presentations and pathological observations of non-neoplastic injuries among smokers frequently do not fit into what has been previously described as a single disease entity or diagnosis but demonstrate shared features. This reflects 1) temporal disparities with the patient serially exposing lung tissue to CSP over decades, 2) differences in the deposition and retention of particle which is heterogeneous, and 3) progression of injury. Accordingly, smoking-related injury consists of sequelae to particle exposure that overlap and coexist with one another (eg,

Correspondence: Andrew J Ghio
Human Studies Facility, US Environmental Protection Agency, 104 Mason Farm Road, Chapel Hill, NC, USA
Email ghio.andy@epa.gov

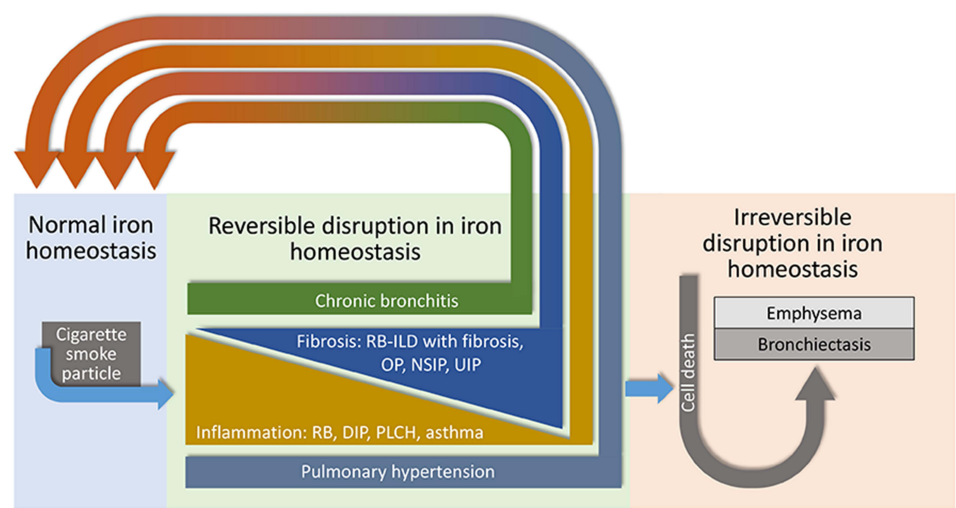


Figure 1 Schematic depicting the mechanistic basis for non-malignant lung injuries with smoking. CSP disrupts cell iron homeostasis by complexation and sequestration of intracellular metal. Host responses of inflammation, mucus production, and fibrosis attempt to reverse inadequate cell iron concentrations. Without correction, the deficiency in cell iron leads to irreversible cell death evident in emphysema and bronchiectasis. RB, respiratory bronchiolitis; DIP, desquamative interstitial pneumonitis; PLCH, pulmonary Langerhans' cell histiocytosis; RBILD, respiratory bronchiolitis with interstitial lung disease; OP, organizing pneumonitis; NSIP, non-specific interstitial pneumonitis; UIP, usual interstitial pneumonitis.

asthma-COPD and combined pulmonary fibrosis and emphysema syndromes) (Figure 2).

Smoking and Iron Homeostasis

During smoking, a lack of oxygen and an incomplete incineration of tobacco result in the generation of

a complex aerosol, which includes condensed liquid droplets (the particulate fraction or tar) suspended in a mixture of volatile/semi-volatile compounds and combustion gases (the gas fraction). Smoking one cigarette exposes the human respiratory tract to a remarkable mass of particulate matter (PM), between 15,000 and 40,000

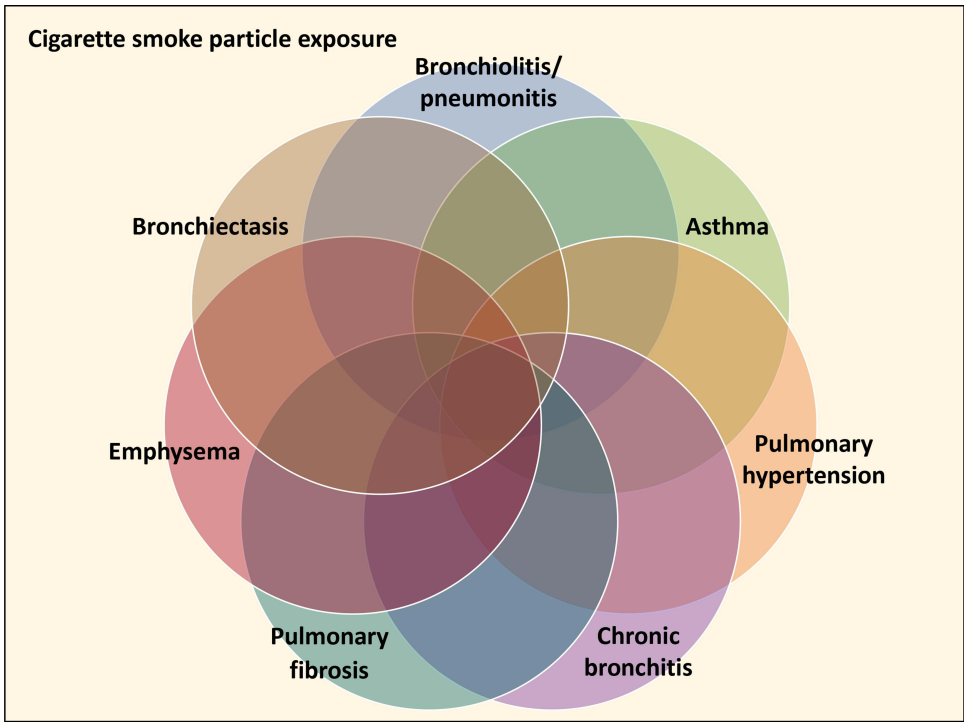


Figure 2 Venn diagram of potential non-malignant lung injuries after cigarette smoking. Presentations with overlap between two or more lung injuries are numerous.

μg .¹ The deposition fraction of CSP (with a mean diameter of about 0.2–0.5 μm) in the lung is projected to be 70–90% with the greatest of this occurring in the 16th to 19th generations of airways (which includes the respiratory bronchioles).^{2–5} There is a gravitational gradient in the ventilation distribution with dependent regions receiving more of each breath than the non-dependent regions and particle deposition is subsequently highest in the lower lung lobes.⁶ Soluble components of CSP can be transported to the blood.² Clearance of the insoluble components of the particle is to the gastrointestinal tract and lymph nodes and slowest from the alveolar region where it is dependent on macrophage function.^{7,8} Accordingly, the lungs of cigarette smokers reveal enormous numbers of both intracellular and extracellular particles, with the former most frequently being in macrophages (Figure 3). Particle is observed to fill macrophages in small airways and alveolar regions in the inflammatory response. In the alveolar regions, pigment-laden cells may no longer be evident, but the accumulated particle remains intact with clusters of anthracotic material being discernable and the size of the individual aggregates of particle and the spatial relationships between them approximate those in the cellular collections.

Cells exposed to tobacco smoke become laden with a material that is variably described to be orange, red, brown, or black in color and fluorescent. This appearance is consistent with humic-like substances (HULIS) which are complex, organic, macromolecular compounds that comprise approximately 7–10% (mass/mass) of CSP.^{9–18} As a result of having a variety of oxygen-containing functional groups (mostly phenols and carboxylates), HULIS reacts with metal cations to form coordination complexes and, among these, iron is kinetically the most favored.^{19–22} Subsequently, cell exposure to CSP, with its included HULIS, results in an internalization and successive complexation and sequestration of host iron at the particle surface.^{12,23–29} Compounds which complex iron can provide a template for additional condensation of iron-hydroxides and a formation of oxide-centered nanoparticles follows.³⁰ Iron homeostasis in the cell is disrupted. Macrophages laden with iron (ie, sideromacrophages) are observed with smoking and provide direct support for the capacity of CSP to disrupt iron homeostasis and accumulate metal in exposed cells (Figure 4).^{16,31–33} Quantities of this specific metal in macrophages increase proportionally to the frequency and duration of cigarette smoking.^{25,28,34}

A unique coordination chemistry led to the evolutionary selection of iron for a wide range of fundamental cell

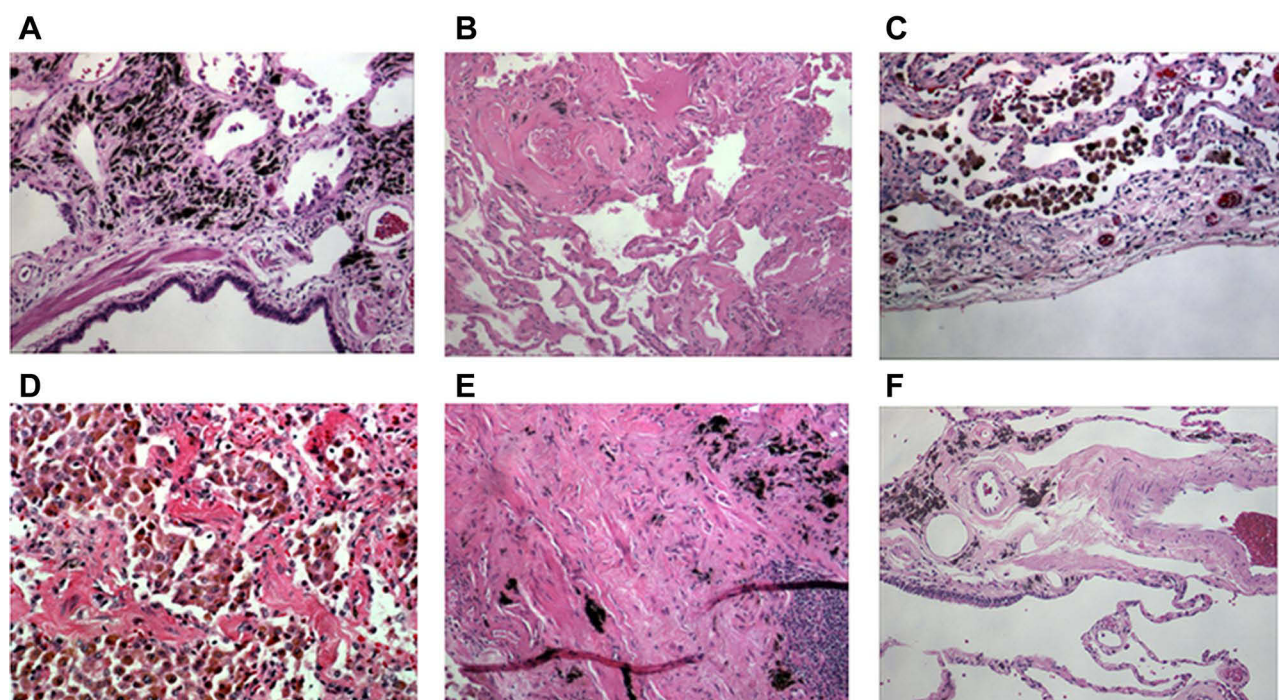


Figure 3 CSP in the human lung. Smokers show particle in close proximity to both airways (A) and vascular structures (B) and intracellularly within macrophages in distant sub-pleural regions (C). CSP is evident in lung resected from patients with inflammatory lung injury (D), fibrosis (E), and emphysema (F). Stain is hematoxylin and eosin. Magnification approximates 100 $\times$.

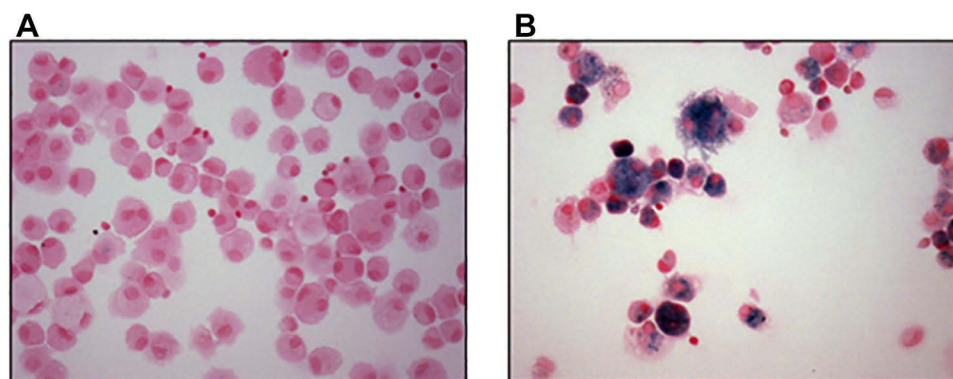


Figure 4 Siderophages in macrophages collected by bronchoalveolar lavage. Macrophages collected from nonsmoker (**A**) do not stain for iron (blue) while those from a smoker (**B**) do. Stain is Perl's Prussian blue. Magnification approximates 400 $\times$.

functions and its availability is essential for almost every form of life.³⁵ A lack of available iron can restrict life in environments ranging in size from the ecosystem of the Pacific Ocean to a bacterium.^{36,37} Iron concentrations inadequate to meet the requirements for life necessitate a development of pathways to acquire the critical metal. Concurrently, a metal-catalyzed generation of radicals presents a potential for oxidative stress. Therefore, iron homeostasis, including its import, storage, and export, are vigilantly regulated and life exists at the interface between iron-deficiency and -sufficiency. After the cell reaches some lower threshold of iron concentration, there is an

obstruction of the cell cycle and an initiation of regulated cell death.^{38–40}

Intracellular iron levels immediately available for complexation in lung cells exposed to CSP are very low approaching the concentration of the labile iron pool (less than 1–5 μ M).^{41,42} Accordingly, CSP competes for iron utilized by the cell for functions frequently critical for survival (Figure 5). With metal accumulation by the particle surface following its complexation, the cells exposed to CSP must appreciate a functional iron deficiency. While the total iron is either the same or increased, the concentration of metal in the cells exposed to CSP will be

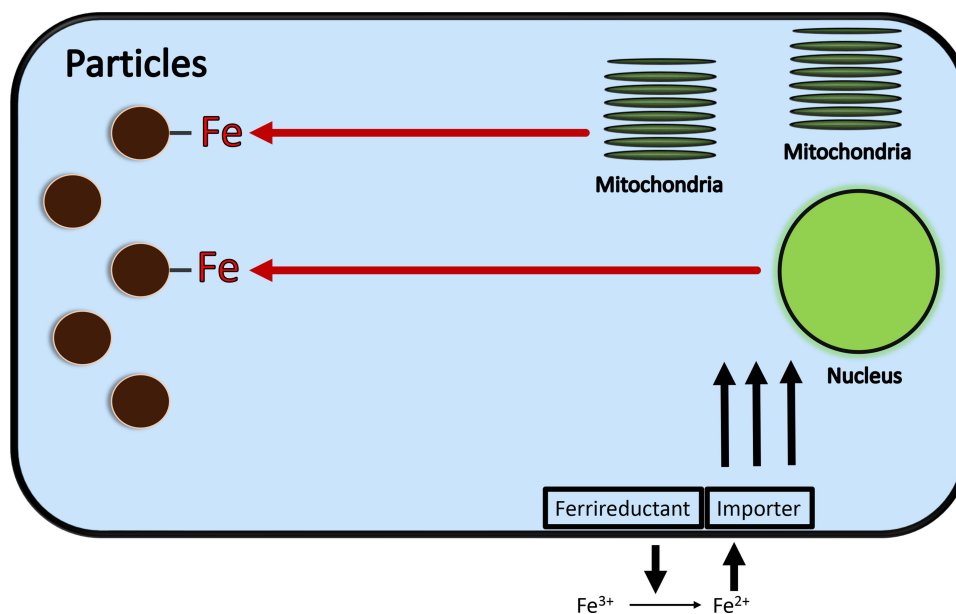


Figure 5 Schematic for changes in iron homeostasis following CSP exposure. Functional groups at the surface of the CSP, including HULIS, complex and sequester iron from the cell. A functional metal deficiency results. In response to a reduction in intracellular iron, the cell upregulates iron import in an attempt to reacquire requisite metal. If the cell response to increase metal is inadequate, function and survival are compromised.

insufficient to meet the requirements for function as a result of its sequestration by the particle. The cell subsequently increases import of iron and, if successful, cell concentrations rise and metal levels will be sufficient to meet requirements for continued function despite the complexation of host resources by internalized CSP. However, if either enough host iron is complexed by the particle or the cell response to increase metal is inadequate, function and survival are compromised and cell death is predicted.

Investigation employing bronchoalveolar lavage supports both increased and decreased iron concentrations in the epithelial lining fluid of smokers and will be dependent on whether the individual is actively smoking.^{27,43,44} Exhaled breath condensate (EBC) is an alternative approach to sampling the epithelial lining fluid and has demonstrated decreased iron concentrations among patients diagnosed with smoking-related lung disease (eg, COPD).^{44–46} After complexation of iron by the CSP affecting a functional iron-deficiency, the cell will attempt to reverse the loss of requisite iron and this will include expression of proteins involved in metal import (eg, transferrin receptor and DMT1).^{47–49} Compared to nonsmokers' lungs, both transferrin and transferrin receptor expression in cells and fluids can be higher relative to those with smoking-related disease. Iron is imported into a cell only after ferriredoxin (eg, superoxide generation) and this must similarly increase with exposure to CSP.⁵⁰ In an animal model exposed to cigarette smoke, increases in lavage concentrations of iron and ferritin, and nonheme iron concentrations in the lung are reversed after filtering to remove particles.²³ Exposure of respiratory epithelial cells to CSP (and its components) affects an increased import and accumulation of iron.^{14,23} These findings support complexation of cell iron by CSP and a cellular response to a functional metal deficiency in the lungs of smokers and/or those with disease after smoking.⁵¹

Iron homeostasis is also altered systemically with smoking. Reflecting a diminished availability of metal after smoking, iron deficiency is common in smoking populations.^{52–54} Anemia is more frequently observed in both smokers and populations with smoking-related disease with a prevalence in COPD that varies between 4.9% and 44%.^{52,55–58} The two primary types of anemia seen in smoking-related disease are anemia of chronic disease and iron-deficiency anemia; both are related to alterations in iron homeostasis.

Bronchiolitis/Pneumonitis

Respiratory bronchiolitis (RB) is a universal inflammatory reaction that occurs in smokers (ie, smoker's bronchiolitis).^{59,60} It is characterized histologically by a patchy accumulation of pigmented smoker's macrophages, consistent with an accrual of HULIS, in the respiratory bronchioles (ie, that region with the greatest CSP deposition).^{61,62} RB is most frequently noted as an incidental histologic abnormality and few patients are symptomatic. With continued exposure, greater particle deposition will occur in the alveolar region where it will initiate an inflammatory response consistent with desquamative interstitial pneumonitis (DIP) which is characterized by numerous pigmented macrophages, and sometimes giant cells, within the distal airspace of the lung (Figure 3D).^{63,64} The prevalence and incidence of DIP are unknown, but an association with cigarette smoke has been demonstrated and the majority of patients are smokers.⁶⁵ While the feature that differentiates RB from DIP is most frequently cited as the distribution and extent of macrophage accumulation (that is bronchiolocentric in RB and more diffuse in DIP), there are no reliable histologic features to distinguish the two inflammatory responses with certainty and they can be considered different phases of a single response.^{60,66} Smoking cessation is currently considered the primary treatment for both RB and DIP and survival rates are favorable. This inflammatory response can persist in patients after smoking cessation supporting particle participation as CSP remains in the lung for years.^{67,68}

Pulmonary Langerhans' cell histiocytosis (PLCH) is characterized by the proliferation of specialized dendritic cells, known as Langerhans' cells, that form multiple, bilateral, stellate-shaped nodules, which frequently cavitate.^{69,70} These peribronchiolar lesions are often associated with smokers' pigmented macrophages as well as an influx of eosinophils and lymphocytes in the distal bronchioles.^{61,70} The incidence of PLCH is unknown and is difficult to determine since it is frequently asymptomatic and can resolve spontaneously. PLCH most frequently occurs in young adults with a peak incidence at 20–40 years of age, and almost all patients are either current or previous cigarette smokers. With smoking, a mixture of respiratory bronchiolitis, DIP, and PLCH is commonly observed histologically and their differentiation from one another can be difficult.⁶⁶ Since the CSP deposition is greatest at the respiratory bronchiole, RB is the earliest

inflammatory injury observed after smoking and temporally this can be followed by DIP and PLCH as the exposure increases with continued smoking.

The focus of the inflammatory response after smoking is suggested to be a reacquisition by the host of its iron sequestered by CSP. This is equivalent to inflammation following exposures to microbes, which reduces infection to a “battle for iron”.³⁶ By impacting a functional iron deficiency in the host, CSP exposure initiates inflammation similar to other particles.^{71–84} This response includes increased oxidative stress, cell signaling, transcription factor activation, and release of mediators.^{83–91} Cell exposures to compounds and substances with a capacity to complex iron, including CSP and other particles, correlate with increased oxidant generation.^{92–95} Such oxidants, specifically superoxide and its related products, can follow a loss of requisite iron from the cell to a particle.⁷⁹ At the level of a living system, the provision of an iron-deficient diet results in an anemia and an increase in oxidant generation.⁹⁶ Superoxide, produced by the cell in response to metal deficiency, enables the import of requisite iron through the chemical reduction of Fe^{3+} to Fe^{2+} . This ferrireduction is an essential, and frequently limiting, reaction in iron import.^{97–99} Cellular iron deficiency and its associated oxidative stress influence numerous pathways which coordinate an inflammatory response including an activation of specific kinases (eg, p38, JNK, ERK1, and ERK2).^{87,88,100–105} Phosphorylation of kinases following exposure to inflammatory agents, including particles, can be decreased by augmenting the cell concentration of available iron supporting a role in metal homeostasis.⁷⁹ Comparable to kinase activation, diminished cell iron concentration corresponds to an activation of specific proinflammatory transcription factors also favoring a participation in metal homeostasis (eg, NF- κ B, AP-1, HIF- α , CREB, and NRF).^{87,88,101,103,106–116} Finally, inflammatory mediator release increases with a decreased availability of iron.^{83,85,100,101,107,113,115,117,118} Cigarette smoking demonstrates a dose-dependent association with increased concentrations of inflammatory cytokines and cells in the lower respiratory tract.¹¹⁹ This association of the release of pro-inflammatory cytokines with a disruption in iron homeostasis has been demonstrated after exposure to CSP.^{14,81,120–122} This coordinated inflammatory response to CSP is comparable to activation of pathways observed with metal deficiency following cell exposures to other compounds and substances with a similar capacity to complex iron.^{83,85,89} Even with

cessation of smoking, the HULIS in the retained particle will continue to complex available host iron as long as it persists in the lower respiratory tract therefore impacting a functional metal deficiency and initiating the inflammatory response (eg, RB and DIP).

Asthma

There are numerous associations between asthma, an inflammatory airways injury, and smoking.^{123,124} Both current and former smokers are at increased risk of developing asthma.^{5,125–127} Smoking also increases exacerbations, severity, hospitalizations, and mortality of asthma.^{5,125,127–131} Asthma associated with smoking demonstrates poorer control, impaired response to corticosteroid therapy, accelerated decline in lung function, and increased rate of healthcare utilization.^{123,124,128} There is a comparable causal relationship between exposure to environmental tobacco smoke (ETS) and asthma in non-smoking adults and children.^{126,132–138} Evidence also supports an association between ETS exposure and asthma exacerbation.¹³² Bronchial hyperresponsiveness (BHR) is regarded as a hallmark feature of asthma and bronchoprovocation testing is performed to support its diagnosis.¹³⁹ Both cigarette smoking and ETS increase BHR.^{140–146} Finally, there is an interaction between maternal smoking and asthma with increasing risk for physician-diagnosed asthma in the newborn and during both childhood and adolescence.^{136,147–151}

The mechanism by which exposure to cigarette smoke causes these effects is not established but asthma can be associated with a deficiency of iron (both functional and absolute) initiated by particle exposure.¹⁵² While the largest quantity of CSP can be observed in the distal alveolar regions with smoking, the highest particle mass per epithelial surface area can be in the proximal conducting airways, due to smaller surface areas.⁹ Accordingly, an associated functional iron deficiency by the CSP and initiation of oxidative stress, activation of cell signaling and transcription factors, and release of proinflammatory mediators can result in an airways inflammation recognized clinically as asthma. In support of this, the inhalation of an iron chelator (ie, citric acid) increases BHR and causes bronchoconstriction.^{153,154} In addition, particle-related exposures other than cigarette smoking, which similarly complex metal and disrupt iron homeostasis, elevate BHR in a dose-dependent manner.^{145,155–157} Regarding maternal smoking and asthma in the newborn, iron is accumulated by the developing fetus against

a concentration gradient and many stresses will impact the availability of this metal. There are statistically significant negative correlations between maternal smoking and an infants' total body iron.¹⁵⁸ The number of cigarettes smoked per day by the mother correlates negatively with iron availability in newborn infants. In addition, maternal smoking during pregnancy decreases the concentration of available iron in both umbilical cord blood and placenta.¹⁵⁹

Pulmonary Hypertension

Pulmonary hypertension (PH), an abnormal elevation in the pressures of the pulmonary arterial system, is initially an inflammatory disease. Exposure to cigarette smoke directly impacts pulmonary vascular cells to release an abnormal production of mediators, many pro-inflammatory, that control vascular cell proliferation, and vasoconstriction/vasodilatation.^{160–163} Pulmonary arterial intimal thickening and vessel narrowing are early changes in smokers' lungs and these correlate with endpoints of other smoking-related lung injury (eg, severity of bronchiolitis and emphysema).^{164,165} Among smokers, there is also an increase in pulmonary vascular resistance due to vasoconstriction and thickening of the walls caused by proliferation of smooth muscle and other cells (ie, remodeling of the pulmonary vasculature).

PH is associated with a disruption in iron homeostasis and decreased metal availability. In patients with smoking-related lung disease, iron deficiency was associated with an increased systolic pulmonary artery pressure.¹⁶⁶ Iron in lung tissue, which is complexed by CSP, shows an association with right ventricular systolic pressure among patients with idiopathic pulmonary fibrosis (IPF), a smoking-related lung disease.³³ A high prevalence of iron deficiency is present in patients with idiopathic pulmonary arterial hypertension (IPAH) and metal availability corresponded to hemodynamics, functional class/disease severity, and clinical outcome.^{167–170} Similarly, iron deficiency is found in PH patients (38.25%) (with the highest prevalence being in connective tissue disease associated PAH) and is associated with worsened clinical outcome.^{171,172} The effect of hypoxia on pulmonary arterial pressure can depend on the iron status possibly acting through the transcription factor hypoxia-inducible factor.^{173,174} Extrapolating from chronic left heart failure where iron deficiency is recognized to be common and parenteral iron can change exercise capacity and functional class, treatment of metal deficiency can improve

PH.^{168,169,174,175} After administration of intravenous iron, the rise in pulmonary artery pressures with hypoxia can similarly be attenuated.¹⁷⁶

Regarding a causative relationship of disrupted metal homeostasis with PH, iron is a major participant in pulmonary vascular homeostasis. Intracellular iron deficiency alters pulmonary vascular function and iron-deficient rats can exhibit raised pulmonary artery pressure and right ventricular hypertrophy with profound pulmonary vascular remodeling (eg, prominent muscularization, medial hypertrophy, and perivascular inflammatory cell infiltration).^{177,178} In another animal model, decreased iron availability leads to muscle remodeling, which can be reversed by replacement of the metal.¹⁷⁹

Chronic Bronchitis

Chronic bronchitis (CB) is diagnosed in patients with a cough productive of mucus for at least 3 months per year for 2 consecutive years. The primary mechanisms responsible for excessive mucus are overproduction/hypersecretion, by goblet cells and glands, and decreased elimination. Smoking is the primary risk factor for CB.¹⁸⁰ The diagnosis of CB affects outcomes including lung function, exacerbations, hospitalizations, and mortality.

Mucus production in the smoker's airways is proposed as a host response to reverse the functional iron deficiency resulting from metal complexation and sequestration by CSP. Epithelial interfaces exposed to external environments are dominated by linear polysaccharides with large molecular weights.¹⁸¹ Among these, glycosaminoglycans (GAGs, also called mucopolysaccharides) are a major component of mucus, produced by either epithelium or glands, and composed of amino sugars and uronic acids (all except for keratin sulfate have glucuronic acid or iduronic acid).^{182,183} These sugar acids contain carboxylate and hydroxyl functional groups, negatively charged molecules, which can participate in cation exchange and metal complexation.¹⁸⁴ Reflecting this reactivity, hydroxycarboxylates such as GAGs and other sugar acids complex iron and stain with colloidal iron.^{185–192} In this complexation, these polysaccharides employ both carboxylate and hydroxyl groups in the binding of metal.^{30,193–204} Among the metals, complexation of iron is preferred with the stability constant approximating 10^4 M^{-1} .^{202–204} By acting like organic ligands, these polysaccharides participate in iron homeostasis to enhance bioavailability of metal thus increasing import.^{205–210} Subsequently, production of these polysaccharides can reverse a functional iron

deficiency. The ability of these polyanionic polysaccharides to complex metals can include bridges between the polymer chains which lead to the formation of ionic cross-linked, supermolecular structures and these can modify the gel polymer networks.^{211–216} Subsequently, with binding of available metal, there is crosslinking, water is expelled, the viscosity of the polysaccharide matrix is altered, chain stiffening follows, and mechanical properties can be changed.^{217,218} Metal cations, including iron, can also depolymerize polysaccharides comparable to other biopolymers.^{219–223} The activity of a lyase can be increased and decreased after exposure to specific metals.^{224,225} With iron deficiency, lyases are activated to produce oligomers (eg, oligogalacturonides) which demonstrate important bioactivities including metal uptake (Figure 6).^{226,227} In contrast to iron deficiency, increased metal availability diminishes this lyase activity.²²⁸ Accordingly, low iron availability in microbials triggers a coordinated expression of genes encoding lyases with iron transport functions.^{229,230} This synthesis and depolymerization of polysaccharides provides polymeric units with a high number of binding sites utilized for metal import.²³¹ This is comparable to several protein polymers which are utilized to increase metal uptake after proteolytic degradation and complexation of the metal by fragments with decreased molecular weight, increased charge with disclosure of reactive functional groups.²³²

In support of a role for these polyanionic polysaccharides in human iron homeostasis, other living systems utilize them to acquire metal.^{233–235} Similar to mucus, capsular polysaccharides in microbes have abundant uronic acid subunits that participate in metal uptake.^{210,236,237} Among the metals, uptake by a capsule is greatest for iron and large concentrations can be detected.^{210,238} Iron availability influences both formation of a capsule and the production of these polysaccharides.^{239–243} Microbials can also generate biofilms, which include polyuronates of varying chain length and composition.^{196,244} With biofilm formation, microbes effectively concentrate and utilize metals with iron being preferred over others.²⁴⁵ Microbes respond to iron deficiency by using the metal complexed by the components of the biofilm as a “sink”. Removal of iron from a medium increases biofilm and polyuronate production as the microbe attempts to reverse the deficiency.^{246–248} In contrast, elevated iron concentrations inhibit biofilm formation in a dose-dependent manner.^{249–254} Accordingly, biofilm production is induced in iron-restricted conditions and is repressed by increased availability of the metal.²⁴⁹ Polyuronates, and mucus production, also participate in iron uptake in plants and animals.^{229,230} A large proportion of metal-binding, required for iron transport in the gastrointestinal tract, is found in goblet cells and the mucin layer located extracellularly in the lumen.²³¹ Mucus can be demonstrated to have metal-binding activity using

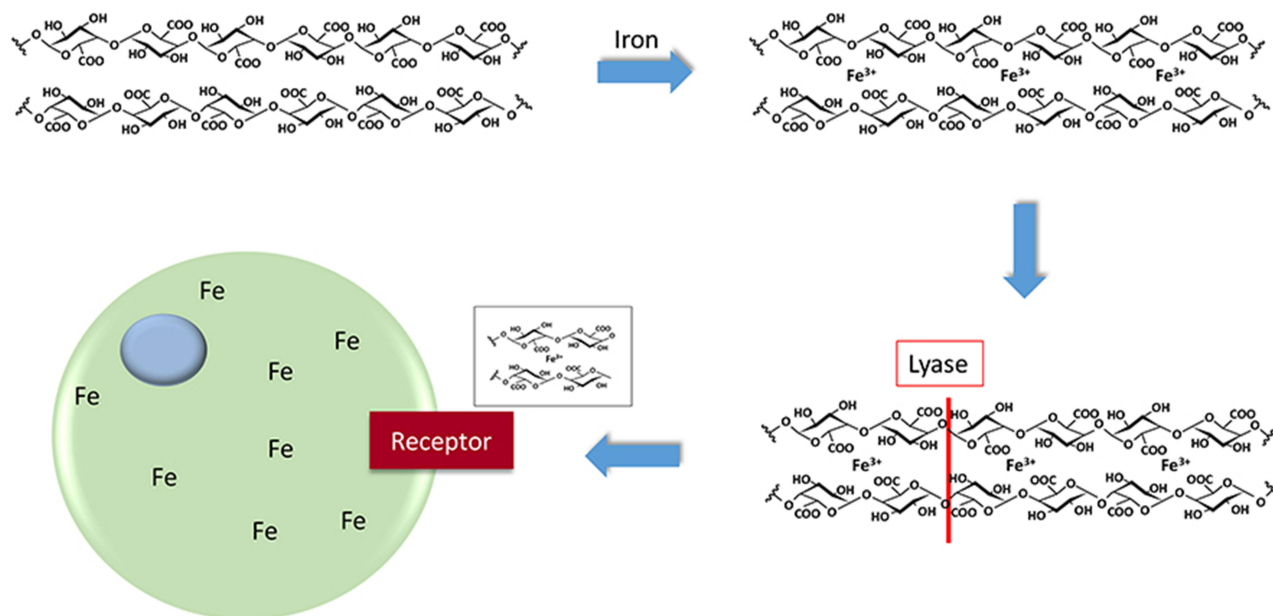


Figure 6 Schematic of iron import by polyanionic polysaccharide. Iron is bound by the polysaccharide using moieties such as carboxylates and hydroxyl groups. Lyase activity, increased by the metal deficiency, provides oligomers with bound iron to the cell for receptor-mediated uptake.

histologic methodology.^{190,191,255,256} Colloidal iron stains are employed as an assay for in situ iron binding capacity in mucus and confirm metal-binding by carboxylated and sulfated mucopolysaccharides and glycoproteins.^{257–260} Following phagocytosis of mucus, macrophages demonstrate an accumulation of iron.²⁶¹ All this evidence supports an increased mucus production in the smoker's airways as a beneficial host response, which participates in reversing a functional metal deficiency following complexation of iron by CSP. The inclusion of polyanionic polysaccharides (eg, polyuronates) in mucus effectively positions a negative charge on human respiratory cell membranes in the airways which complexes iron. After binding the metal, the polysaccharide is acted on by lyases providing a continual supply of a low molecular iron chelate facilitating import and increasing availability.

Pulmonary Fibrosis

Evidence supports a relationship between smoking and pulmonary fibrosis.^{59,262–277} This pathobiologic process can be characterized by excessive extracellular matrix (ECM) production, alveolar epithelial cell loss, and alveolar collapse in response to injury. Radiologically, pulmonary fibrosis is recognized by a combination of reticular opacities, volume loss, traction bronchiectasis (dilated, irregularly shaped airways), and honeycombing (clusters of small cysts located in the extreme periphery of the lung), of which the latter two develop as a result of loss in aerated lung volume and permanent alveolar collapse. Pathologically, pulmonary fibrosis manifests as several different histologic patterns: 1) organizing pneumonia (OP), characterized by round or oval pale-staining deposits consisting of fibroblasts, myofibroblasts, collagen, and fibrin within respiratory bronchioles, alveolar ducts and alveoli²⁷⁸; 2) nonspecific interstitial pneumonia (NSIP), characterized by inflammation and/or fibrosis in the lung interstitium occurring in a spatially homogeneous pattern and with preservation of overall lung architecture²⁷⁹; and 3) usual interstitial pneumonia (UIP), the most severe form of lung fibrosis, characterized by heterogeneous areas of dense fibrosis interspersed with areas of relatively normal lung architecture, fibroblastic foci, and honeycombing.²⁸⁰ With the deposition and retention of CSP being greatest in the region of the respiratory bronchioles, an initial fibrosis evolves from the inflammatory response, correlates with the amount smoked, and is observed pathologically as a peribronchiolar fibrosis.^{4,59,70,281,282} With continued smoking (ie, CSP exposure), deposition will extend

distally beyond the respiratory bronchiole. OP and NSIP represent earlier phases of the tissue response to CSP while UIP represents a lung response to higher doses and subsequently a later stage. One histologic presentation of injury may progress into the next with overlapping patterns being observed which make strict histopathologic diagnosis difficult or impossible.^{59,283,284} OP components are common lesions in both NSIP and UIP cases, and as fibrosis evolves, pathological areas of NSIP are observed with UIP.^{285–290}

Below the resolution of any radiologic imaging, fibrosis can be observed in a majority of smokers.^{291–293} In the last two decades, fibrotic lung injury has presented radiologically without clinical symptoms; studies on large cohorts have reported such interstitial lung abnormalities (ILAs) in 8–20% of the smokers undergoing high-resolution computed tomography (CT) scanning.^{267,294–296} On pathological examination, ILAs can include both inflammation and fibrosis.²⁹⁷ ILAs are a precursor to clinically evident smoking-related fibrotic lung disease.

In support of a role for a metal deficiency participating in lung fibrosis, animal models of fibrotic injury utilize compounds and substances which complex and sequester iron (eg, bleomycin, asbestos, silica, and paraquat).²⁹⁸ Reflecting a metal deficiency, 1) iron is lower in breath condensate and 2) gallium uptake, indicating transferrin-mediated metal uptake and demand for metal, is increased in the lungs of patients with fibrosis.^{299–301} In patients with particle-related fibrotic lung disease and animal models of lung fibrosis, macrophages demonstrate increased transferrin receptor (CD71) indicating iron insufficiency.^{302,303} A humic substance (fulvic acid), which is chemically analogous to HULIS in CSP, stimulates collagen secretion by articular chondrocytes.³⁰⁴ Finally, increased metal availability after treatment with ferric citrate decreases fibrosis in a non-pulmonary tissue of an animal model.³⁰⁵

Although pulmonary fibrosis is detrimental to the patient from a lung mechanics and gas exchange standpoint, the fibrotic process may benefit the host response to functional iron deficiency associated with the loss of metal following complexation by CSP.³⁰⁶ Fibrotic lung injury associated with smoking is characterized by an excessive accumulation of ECM. Comparable to mucus, polyuronates are a major component of both GAGs and ECM. Hyaluronic acid (HA) is the most abundant GAG in ECM and is increased with smoking. Comparable to other polyuronates, it forms a coordination complex with transition metals including iron.^{307–309} Metals participate in the

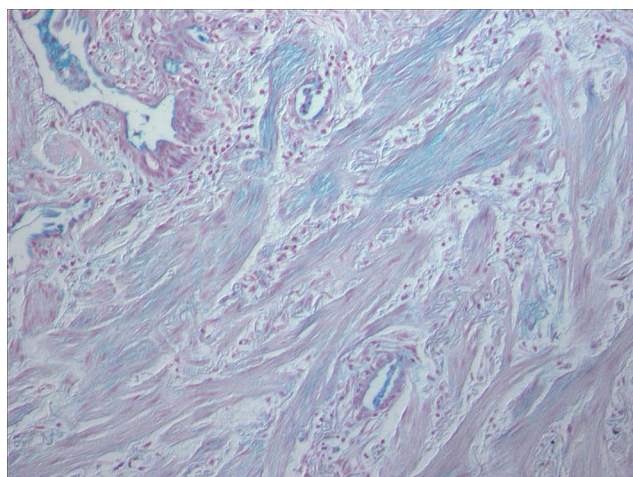


Figure 7 ECM components bind iron. Collagen demonstrates staining for iron (blue) supporting a role for uptake. The stain is colloidal iron. The magnification approximates 100 $\times$.

depolymerization and, following the reaction with iron, HA will be degraded.^{307,310,311} Such depolymerization of GAG polysaccharides to oligosaccharides improves cell import of metal. Increased availability of iron inhibits hyaluronidase and degradation products of HA function as mediators of inflammation.^{312–314} The cell receptor for HA is regulated by metal availability and is a major participant in iron uptake.³¹⁵ Collagen is another major component of ECM, being the most abundant protein in mammals, and is also increased in smokers' lungs.^{268,316–323} As a result of abundant functional groups including carboxylates, hydroxyls, and amines, collagen complexes iron which may increase cell availability and possibly reverse a deficiency (Figure 7).^{324–327} Collagen peptides demonstrate iron binding activity.³²⁸ This interaction of collagen with metals is a recognized method for its stabilization (ie, tanning which most commonly is achieved with chromium but iron cations can be employed).^{329,330} The metal complexed to collagen is considered available to the host and higher doses of iron decrease collagen synthesis further supporting a role in metal homeostasis.^{331–333} Relationships between other components of ECM and iron homeostasis are also evident.^{268,334–336} A Gamma-Gandy body, with dense fibrous tissue and collagenous fibers encrusted with iron and calcium, demonstrates the capacity of ECM to complex metal cations and impact their homeostases.³³⁷

Emphysema and Bronchiectasis

Emphysema is characterized by abnormal, permanent enlargement of the airspaces distal to the terminal bronchiole accompanied by destruction of their walls. The early

lesion, focal emphysema, involves the branches of the terminal bronchiole, and this is followed by microscopic emphysema with passages of airways affected being the two distal orders of respiratory bronchioles or the first order of alveolar ducts (ie, the site of greatest CSP deposition). Centrilobular emphysema following smoking originates in areas of “parenchymal soot deposits”, immediately adjacent to retained particle, and severity is dependent on the total quantity of CSP (Figure 8).^{23,338–344} In these emphysematous foci, there is a brownish-colored pigment noted in both macrophages and interstitium reflecting HULIS in CSP. This relationship between CSP and emphysema suggests particle participation in the destructive process responsible for tissue injury.

Positive stains with Perls' Prussian blue and accumulation of ferritin and hemosiderin demonstrate a disruption of iron homeostasis in emphysema.^{23,345} In mice, provision of an iron-depleted diet was associated with more severe emphysema following cigarette smoke exposure reinforcing the role of the functional metal deficiency in pathogenesis.³⁴⁶ When successful in resolving the functional iron deficiency following complexation of host metal to the reactive functional groups at the particle surface, inflammation and fibrosis can reverse.^{347–352} However, with failure to resolve an insufficiency of requisite iron, the cell cycle is obstructed and a form of regulated cell death is initiated.³⁵³ An increase in apoptotic



Figure 8 The correlation between CSP and emphysema. Lung tissue of a smoker exhibits a close association between CSP and emphysematous destruction of the parenchyma (ie, bullous formation). The magnification approximates 10 $\times$. Adapted with permission of the American Thoracic Society. Copyright © 2021 American Thoracic Society. All rights reserved. Ghio AJ, Hilborn ED, Stonehuerner JG, et al. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med*. 2008;178(11):1130–1138.²³

cells is observed in the lungs of emphysematous patients.^{354–370} Since this is not counterbalanced by an increase in proliferation, there is a loss of cells resulting in cell/tissue destruction. While mechanisms involved in cell death with emphysema can include a wide range of mediators (eg, oxidative stress, ceramides, vascular endothelial growth factor, and inflammatory cytokines), the critical factor is iron availability. As a result of its central role in complexing the metal and determining availability, cell death will be initiated immediately adjacent to the particle (ie, focal and centrilobular emphysema in the distal bronchiole) as this corresponds to those cells that are functionally iron deficient. Exposure to a substance with this same capacity for metal complexation results in a comparable sequestration of iron, cell death, and contiguous tissue destruction.^{371,372}

Bronchiectasis is defined as a permanent enlargement of airways in the lung. It is commonly observed in smoking populations and in IPF patients, with prevalence that can approach 50% and 95%, respectively.³⁷³ The deposition of CSP per unit surface area of a proximal bronchus can be considerably elevated relative to that calculated for the whole lung and airway epithelial cell exposure can be equivalent to or greater than that of alveolar epithelial cells.³⁷⁴ Bronchiectasis can result from the disruption of iron homeostasis by CSP in airways with a functional iron deficiency associated with smoking blocking the cell cycle and impacting cell death in airway epithelial cells comparable to that in alveolar epithelial cells observed in emphysema. However, apoptosis in large, tubular structures (eg, bronchi and arteries) produces a pattern of injury, which can include widening and distortion of the cylindrical tissue organization.^{375–378} Accordingly, after some threshold of cell death is realized in the airways, widening (that is bronchiectasis) is anticipated. In support of this relationship, indices of emphysema (the other lung injury involving significant cell death after CSP) correlates with bronchiectasis.³⁷⁹ In addition, the remodeling of lung tissue in UIP after smoking includes a continuum of injury, which includes “traction” bronchiectasis and honeycombing. Rather than distinct entities, bronchiectasis and honeycombing reflect a spectrum of injury with the latter representing the final product after exposure of the airway to CSP. In support of this, radiographic findings typical of honeycombing and respiratory-lined cysts correspond closely with bronchiectasis histologically and they represent dilated bronchioles and alveolar ducts with apoptotic cells.^{59,380,381}

COPD

COPD is considered to be one of the most common respiratory diseases and a leading cause of death in the world. It is defined physiologically by airflow limitation that is not fully reversible. Most commonly, COPD is considered to include some combination of CB and emphysema.^{180,382–384} Cigarette smoking is the major risk factor for developing COPD with up to 90% of the diagnosed patients being ever-smokers. Smoking cigarettes decreases all indices of lung function but particularly affects flows.^{385,386} The greater the number of cigarettes smoked, the faster the rate of decline in lung function and losses can be extreme in COPD patients.³⁸⁶

Associations of pulmonary function tests, blood and bronchoalveolar lavage endpoints, and health statistics with indices of iron homeostasis support an involvement of the metal in COPD pathogenesis. Spirometric measures can correlate positively with serum ferritin and iron concentrations as well as transferrin saturation.^{387–389} However, there is a negative correlation between FEV₁/FVC ratio and serum ferritin among both smokers and nonsmokers supporting a relationship between airflow obstruction and a disruption of iron homeostasis.^{390,391} Among COPD patients, lower percent transferrin saturation can be associated with worsening dyspnea and serum iron concentrations, transferrin saturation, hematocrit, and hemoglobin predict survival.^{392–395} Iron deficiency predicts acute exacerbations for COPD, while anemia increases the mortality risk four-fold in such patients.^{53,396} In those with COPD, anemia is an indicator for health care utilization with lower hematocrit being a risk for long-term oxygen use, hospitalization, increased duration of hospitalizations, and hospital readmission after acute exacerbation.^{392,397–399} Correction of iron deficiency improves dyspnea scores in one cohort of COPD patients and use of blood transfusion enables weaning from mechanical ventilation.^{51,400} Serum iron levels also predict susceptibility of individuals to cigarette smoke with low serum iron being associated with a decline in FEV₁.³⁸⁸ Bronchoalveolar lavage iron and ferritin concentrations are higher in COPD patients and in smokers without COPD when compared to nonsmokers, probably reflecting a CSP component(s) in the lavage and metal complexation, and these increase further with exacerbations.⁴⁰¹ Finally, in addition to these pulmonary function tests, blood endpoints, and health statistics, an association between genetic

factors and susceptibility supports a participation of iron homeostasis in COPD pathogenesis (eg, IREB2).^{402–404}

Infections

Smokers exhibit an increased susceptibility to numerous infections. Microbials require iron to proliferate. A pathogen's survival and virulence are directly related to its success in competing for available iron and metal acquisition from the host determines the virulence of microbes.^{405,406} While the pathogen requires iron concentrations that approximate 10^{-6} M for processes critical to survival, this metal is normally available in the host only at 10^{12} -fold lower concentrations. In the lungs of a smoker, there is an increase in total concentrations of iron but a functional deficiency following complexation by CSP. However, microbes can utilize specialized systems (eg, siderophores and receptors that bind host metal transport and storage proteins) to access the sequestered iron. Subsequently, smoking elevates levels of iron available to microbes in the respiratory tract. Accordingly, smoking is the strongest risk factor for pneumonia and its severity.^{407–415} Similar to pneumonia, viral upper respiratory infections are increased among smokers.⁴¹⁶ With cessation of smoking, the risk for these respiratory infections declines but remains elevated in the ex-smoker, relative to the lifetime nonsmoker, because of the persistence of the CSP and complexed iron.⁴¹⁰

Conclusions

Exposure to cigarette smoke produces a functional iron deficiency following complexation of host metal to reactive groups at the CSP surface. A component of the tissue response is focused on reversing the metal deficiency and initially is inflammatory but rapidly proceeds to include fibrosis. Structural remodeling after cigarette smoking (eg, that observed in chronic bronchitis and pulmonary fibrosis) contributes to correcting the functional iron deficiency and can be reversible. However, with significant disruption of iron homeostasis and unresolved metal deficiency, there will be injury associated with cell death, which is not reversible (eg, emphysema and bronchiectasis). The successful inhibition of a host response can potentially augment cell death and dependent injury; truly effective treatments of chronic bronchitis and pulmonary fibrosis may consequently worsen emphysema and bronchiectasis.

Disclosure

The authors report no conflicts of interest in this work.

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