Perls' Prussian Blue Stains of Lung Tissue, Bronchoalveolar Lavage, and Sputum

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ABSTRACT: Perls' Prussian blue (PPB) stain recognizes Fe^{3+} associated with hemosiderin. The employment of this stain in clinical medicine and research has been extensive and novel applications continue to evolve. Ferruginous bodies are intracellular structures in lung tissue, bronchoalveolar lavage (BAL), and sputum that stain with PPB. Inhaled, insoluble, biopersistent particles and fibers are phagocytosed by lung macrophages and thought to be coated, either partially or completely, with an iron-containing protein at the interface forming a ferruginous body. These structures can be categorized as ferruginous bodies having either an inorganic or a carbonaceous core (e.g., asbestos and byssinotic bodies, respectively). In lung tissue, BAL, and sputum, the only cells that stain with PPB are macrophages. These are described as iron- and hemosiderin-laden macrophages and called either siderophages or sideromacrophages. Siderophages can be observed in the lung tissue, BAL, and sputum after various exposures and can also be associated with many different pulmonary and extrapulmonary diseases.

KEY WORDS: iron, lung diseases, macrophages, alveolar, hemosiderin, ferritin

I. INTRODUCTION

In 1867, the German pathologist Dr. Max Perls was the first to use Prussian blue for the histochemical staining of iron. In the Perls' Prussian blue (PPB) stain for iron, ferric ion (Fe^{3+}) is released from cells after treatment with hydrochloric acid and the metal reacts with potassium ferrocyanide to form ferric ferrocyanide, an insoluble bright blue pigment. In the PPB, ferrous ion (Fe^{2+}) does not produce a colored stain. Intracellularly, iron is stored in ferritin, which delivers the metal to meet metabolic requirements.[1] Hemosiderin is an alternate intracellular, iron-storage complex that can consist of damaged/denatured ferritin with iron cores following proteolytic digestion of the protein coat of ferritin.[2] However, hemosiderin formation can also be independent of ferritin.[2] The origin of hemosiderin is not well-defined but it is regarded a waste product.[3] In contrast to iron in ferritin, the metal associated with hemosiderin is not easily available to the cell.[2,4] The PPB stain recognizes Fe^{3+} associated with hemosiderin.

The employment of this stain in clinical medicine has been extensive. In lung tissue, bronchoalveolar lavage (BAL), and sputum, the presence of such staining has sometimes been misinterpreted as definitive evidence of either pulmonary hemorrhage and/or infection.[5,6] We describe those intracellular and extracellular structures and cells in lung tissue, BAL, and sputum that stain with PPB in healthy and diseased individuals. Subsequently, a mechanistic pathway is provided for the formation of these structures and cells. It is proposed that PPB staining by structures and cells is most consistent with an accumulation of iron after a disruption of the homeostasis and sequestration of this metal in a cell.

II. INTRACELLULAR STRUCTURES THAT STAIN WITH PPB (FERRUGINOUS BODIES)

Ferruginous bodies are intracellular structures in lung tissue, BAL, and sputum that stain with PPB.[7] Inhaled, insoluble, biopersistent particles and fibers are phagocytosed by lung macrophages and thought to be coated, either partially or completely, with an iron-containing protein at the interface forming a ferruginous body.[8,9] In the past, ferruginous bodies have been classified as either asbestos or non-asbestos ("pseudo-asbestos") bodies. With the decreased

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use of asbestos-containing products and equipment and the growing recognition of ferruginous bodies associated with other exposures, it is more useful to categorize these structures as ferruginous bodies having either an inorganic or a carbonaceous core (Table 1).

Historically, ferruginous bodies with an inorganic core were the first to be observed and these were asbestos bodies (Fig. 1A).[10,11] The asbestos fiber is covered by either a regularly-segmented or continuous golden-yellow to red-brown coating of iron and protein. The central core of the asbestos body (the asbestos fiber itself) is most frequently thin, straight, and transparent but can, on occasion, be branched and curved. Asbestos bodies form most frequently on fibers longer than 10 micron and those that are thicker.[12,13] Sheet silicates (including talc), aluminum silicates (including kaolinite), diatomaceous earth, erionite, and numerous other silicates form ferruginous bodies that stain with PPB (Table 1).[9,14,15] Fiberglass, glass, refractory ceramic fibers, and silicon carbide fibers can similarly produce ferruginous bodies.[16–21] Metals can be found at the core of a ferruginous body and such structures are associated with work at a site with exposure to that specific metal.[22–24] Exposures to aluminum compounds, stainless steel, titanium oxide, and iron oxides have produced ferruginous bodies.[9] Finally, ash from fuel and burning of leaves have been reported to be associated with ferruginous body formation.[25]

Those structures with a carbonaceous core comprise the majority of ferruginous bodies observed in the general population reflecting the frequency of exposure to particles such as cigarette smoke particle, soot, and wood stove particle (Table 1 and Fig. 1B).[8,25,26] In addition, occupational exposures to coal, cotton fibers ("byssinotic bodies" in mill workers), and synthetic fibers were similarly associated with comparable structures in lung tissue, BAL, and sputum that stain positively for PPB (Fig. 1C).[27–29]

TABLE 1: Particle and fiber exposures associated with ferruginous body formation

Inorganic core
Silicates and silica
Asbestos
Talc/sheet silicates
Kaolinite/aluminum silicates
Diatomaceous earth
Erionite
Silica
Fiberglass/glass
Refractory ceramic fibers
Silicon carbide
Metal compounds and oxides
Aluminum compounds
Stainless steel
Titanium oxide
Iron oxides
Ashes (fly ash from fuel and ash from leaves)
Carbonaceous core
Cigarette smoke particles
Soot
Wood stove particles
Coal dust
Cotton fibers
Synthetic fibers

III. EXTRACELLULAR STRUCTURES THAT STAIN WITH PPB

There are very few extracellular structures in the lung that stain with PPB. Fibrin deposited in the lower respiratory tract of patients with diffuse alveolar damage can stain with PPB (Fig. 2). In addition, aspiration of iron pills was described to result in metal deposition and staining of the extracellular tissues of a bronchus.[30]

IV. CELLS THAT STAIN WITH PPB (SIDEROPHAGES)

In lung tissue, BAL, and sputum, the only cells that stain with PPB are macrophages. These are described as iron- and hemosiderin-laden macrophages and called either siderophages or siderophages. The presence of these iron-laden macrophages in lung tissue, BAL, and sputum can be assessed semi-quantitatively employing two approaches, the Golde score and the

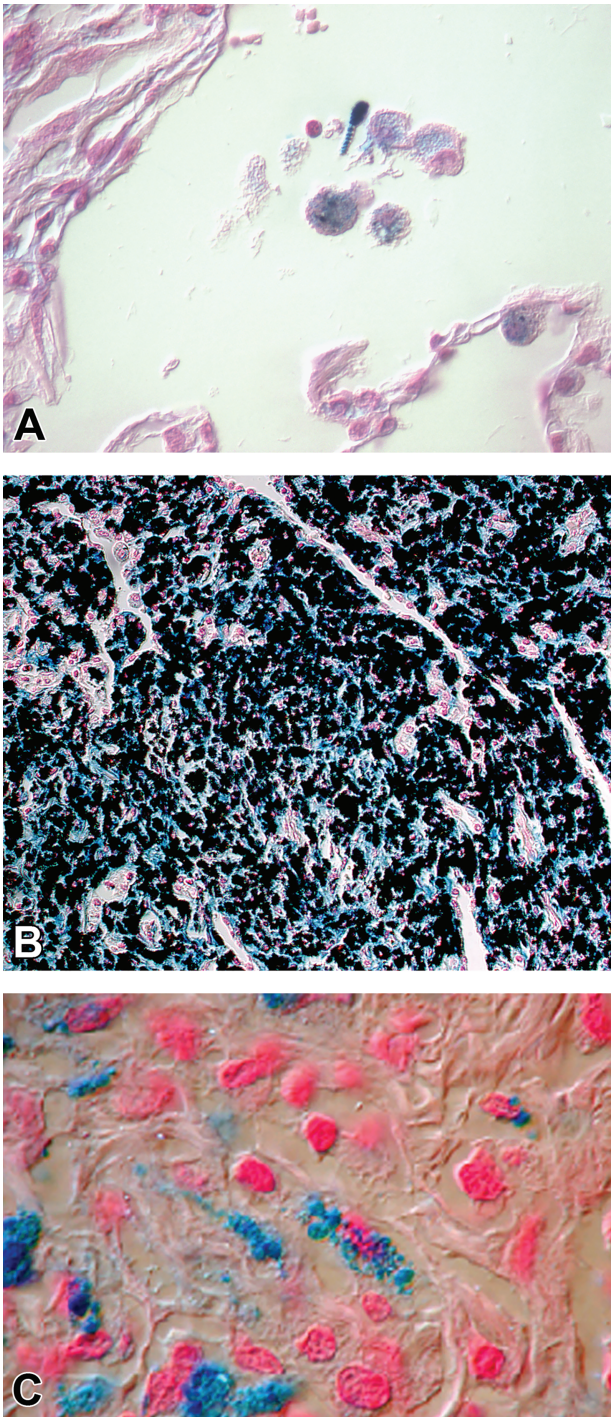

FIG. 1: Ferruginous bodies in human lungs. An asbestos body (A) and ferruginous bodies associated with cigarette smoke (B) and nylon fiber (C) are demonstrated using PPB stain. Magnification approximates 400×, 100×, and 400× in A, B, and C, respectively.

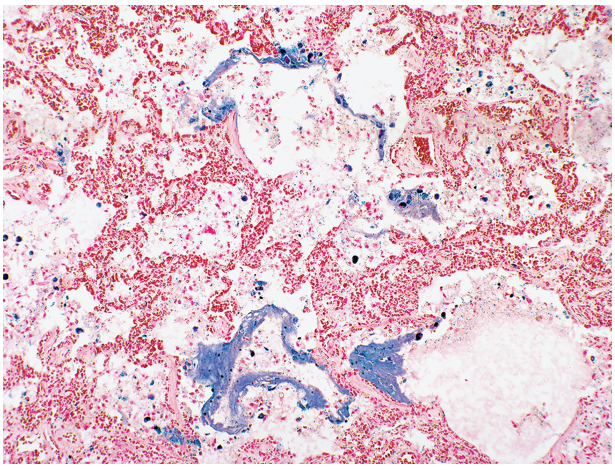

FIG. 2: PPB stain by extracellular fibrin. A patient with diffuse alveolar damage reveals a positive stain for iron corresponding to fibrin localized to the distal respiratory tract. Magnification approximates 400×.

hemosiderin-laden macrophage index. The Golde score assigns a rank to hemosiderin content of 200 macrophages based on a subjective estimate.[31] The hemosiderin-laden macrophage index is the simpler approach and expresses PPB staining as macrophages with any blue granules within the cell after the stain.[32] In respiratory specimens, 200 cells are counted and the hemosiderin-laden macrophage index is reported as a percentage. Siderophages are not normally observed in lung tissue from individuals with no disease but are following specific exposures and diseases. Macrophages in BAL from healthy, unexposed humans can demonstrate positive staining with PPB (these usually comprise < 1–3% of cells) and the number of iron-laden macrophages increase following specific exposures and diseases.[5,33–35] In sputum collected from control populations, siderophage number approximates the same values observed in lavage from healthy subjects and the percentage of hemosiderin-laden cells can be elevated to even greater values with diseases.[36]

Pulmonary hemosiderosis refers to an abnormal deposition of iron in the lung. It is an imprecise term applied most frequently to lung exposures and diseases characterized by an accumulation of macrophages that stain with PPB.

A. Pulmonary Exposures Associated with Lung Siderophages

Comparable to the formation of ferruginous bodies, iron-laden macrophages in respiratory specimens have been most commonly associated with exposures to particles and fibers (Table 2) (Fig. 3A–C). In BAL of exposed individuals, the number of ferruginous bodies correlated with the number of siderophages observed.[37] Smoking was associated with PPB stain-positive macrophages in BAL and sputum.[5,38–41] Exposure to air pollution and burning of biomass increased hemosiderin-laden macrophages in sputum.[36,40,42] Siderophages were demonstrated in both lung tissue collected at autopsy and BAL from patients diagnosed to have asbestosis.[37,43] Coal miners demonstrated significant formation of iron-laden macrophages in lung tissue.[44] Siderophages in sputum were increased in samples collected from particle-exposed individuals including landfill workers, pig farmers, rag pickers, traffic policeman, and railway workers.[45–49] Lung tissue from patients diagnosed with pneumoconiosis (siderosis) after exposure to iron oxide revealed elevated numbers of siderophages.[50] PPB stain-positive macrophages were also noted in a lathe machine worker, in welders, and in others diagnosed with siderosis following iron oxide exposure.[51–54] Lung tissue from Libby Amphibole-exposed rodents similarly showed iron-laden macrophages on PPB staining.[55]

TABLE 2: Pulmonary exposures associated with siderophage formation

Tobacco smoking
Air pollution and biomass burning
Asbestos
Silica
Coal dust
Landfill work, rag picking
Pig farming
Iron oxide
Superparamagnetic iron oxide nanoparticles

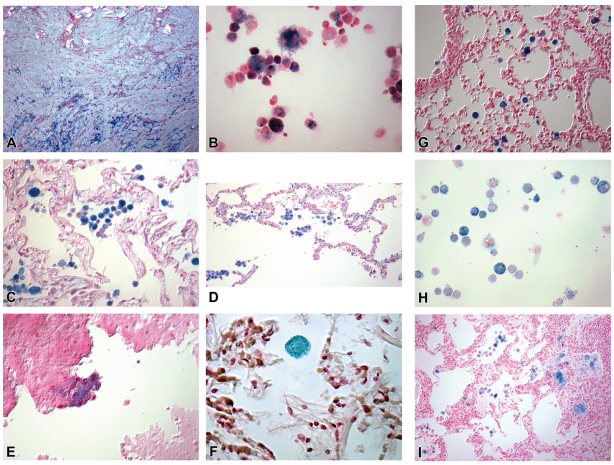

FIG. 3: Siderophages in human lung tissue and BAL. PPB stain reveals iron-laden macrophages in lung tissue following talc pleurodesis (A; magnification approximates 100×), lung tissue from a cigarette smoker (B; magnification approximates 400×), lung tissue after exposure to oil fly ash (C; magnification approximates 400×), lung tissue of patient with infectious pneumonia (D; magnification approximates 400×), lavage from a patient with pulmonary alveolar proteinosis (E; magnification approximates 400×), and lung tissue of patient with lipoid pneumonia and cystic fibrosis (F and G, respectively; magnification approximates 400×). Sideromacrophages are also observed in excised lung tissue and lavage of a rodent model intratracheally exposed to neutrophil elastase (H and I, respectively; magnification approximates 400× and 100×, respectively).

B. Pulmonary Diseases Associated with Lung Siderophages

Siderophages in lung tissue, BAL fluid, and sputum have been reported to be increased in numerous pulmonary diseases (Table 3). Hemosiderin-laden macrophages were noted in specimens of lung tissue and lavage from patients diagnosed with chronic obstructive pulmonary disease and its exacerbation, diffuse pulmonary fibrosis (including idiopathic pulmonary fibrosis), lung cancer, pneumonia, pulmonary alveolar proteinosis, lipoid pneumonia, diffuse alveolar damage, cystic fibrosis, and pulmonary veno-occlusive disease (Fig. 3D–G).[35,56–67] Lung injury after drug abuse was associated with an increased number of siderophages. This included

TABLE 3: Pulmonary diseases associated with siderophage formation

Chronic obstructive pulmonary disease
Diffuse pulmonary fibrosis
Idiopathic pulmonary fibrosis
Lung cancer
Pneumonia
Lipoid pneumonia
Diffuse alveolar damage
Cystic fibrosis
Pulmonary veno-occlusive disease
Injury associated with drug abuse
Transplantation
Lung hemorrhage
Idiopathic pulmonary hemosiderosis
Pediatric
Adult

observations of iron-laden cells in postmortem tissue of abusers and in lavage cells of those smoking crack cocaine.[68–70] In lung transplant recipients, metal accumulation in the lavage cells was observed and the hemosiderin scores increased during the post-operative period.[71,72] Bone marrow transplantation was also associated with elevations in iron-laden macrophages.[73] Both explanted lung tissue and sputum from patients with cystic fibrosis showed increased numbers of iron-laden macrophages.[74,75] In addition, a patient with diagnosed with disseminated hemangiosarcoma provided sputum with iron-laden macrophages.[76] Animals other than humans also demonstrate hemosiderin-laden macrophages with disease. Horses with heaves have significant numbers of siderophages.[77] In a rodent model of airway disease, numerous siderophages were also observed in both the excised lung tissue and lavage following tracheal instillation of neutrophil elastase (Fig. 3H and 3I).[78]

Iron accumulation after pulmonary hemorrhage was quantified using the hemosiderin-laden macrophage index in BAL and these were found to be elevated to some of the highest values reported (i.e., greater than 20%).[79,80] Such hemorrhage was observed in immunocompetent patients, auto-immune mediated lung disease (especially anti-neutrophil cytoplasmic antibody [ANCA]-associated vasculitis and anti-glomerular basement membrane disease), connective tissue disease-associated interstitial lung disease (especially systemic lupus erythematosus), systemic vasculitides, coagulation abnormalities (including primary antiphospholipid syndrome), drug abuse (especially cocaine), inhaled toxins, immunoglobulin A nephropathy exercise, and with thrombolytic therapy.[81–84] The absence of iron-laden macrophages did not exclude pulmonary hemorrhage since there appeared to be some time (several days) required between the actual hemorrhage and the appearance of PPB-positive cells.[5,85,86] Animal models supported a delay in an appearance of hemosiderin-laden cells after exposure to blood.[87,88] Alveolar macrophages from healthy, nonsmoking volunteers demonstrated hemosiderin on PPB staining after exposure to antibody-coated sheep red blood cells but this required 72 hours.[85] The clearance of hemosiderin from the lung after hemorrhage is poorly defined. Investigation has suggested that such clearance may require approximately 2–4 weeks in humans, whereas animal studies supports 7 days to 3 months.[85,87,88]

Finally, idiopathic pulmonary hemosiderosis includes unexplained alveolar hemorrhage with evidence of siderophages. Patients with this disease can present with anemia, diffuse infiltrates on chest X-rays, and hemoptysis. Lung tissue, BAL, and sputum can reveal large numbers of cells that stain with PPB. Idiopathic pulmonary hemosiderosis is a rare disease among pediatric populations and even more uncommon in adults.[32,89–95]

C. Extrapulmonary Diseases Associated with Lung Siderophages

Iron-laden macrophages in the lung can be observed with hematologic, cardiac, gastrointestinal, and rheumatologic diseases and trauma (Table 4). Siderophages were observed in BAL cells among patients diagnosed with thalassemia and receiving transfusions.[96–99] In one study of thalassemic patients who received transfusions, the serum ferritin concentration correlated with PPB staining of the macrophages.[98] In a study on BAL macrophages, one of

TABLE 4: Extra–pulmonary diseases associated with siderophage formation

Heart diseases
Mitral stenosis
Pulmonary edema
Open heart–surgery/cardiopulmonary bypass
Gastrointestinal diseases
Lane–Hamilton syndrome
Heiner syndrome
Hematologic diseases
Transfusion hemosiderosis
Rheumatologic diseases
Rheumatoid arthritis
Systemic diseases
Sepsis
Hemorrhagic shock
Neoplastic diseases
Lymphomas
Hemangiosarcoma (disseminated)
Sudden infant death syndrome, child suffocation, and chronic physical child abuse

the two groups demonstrating the highest number of hemosiderin-laden macrophages was a cohort of patients who had undergone heart transplantation.[5,100]

Heart failure with celiac disease was associated with iron-laden macrophages in a sputum sample.[101] Similarly, animal models of chronic heart failure revealed siderophages in lung tissue.[102,103] Patients undergoing open heart-surgery/cardiopulmonary bypass and those with hemorrhagic shock can show PPB-positive macrophages in tracheobronchial washings.[104,105] In a patient with proven mitral stenosis and iron deficiency anemia, abundant iron-laden macrophages were found in the sputum.[106] A history of myocardial, valvular, or coronary vascular disease with the development of alveolar edema, pulmonary congestion, or acute microscopic lung injury was associated with a presence of siderophages.[107]

Iron-laden macrophages were observed in tissue biopsy and lavage in patients with celiac disease; in a patient diagnosed with Heiner syndrome, a food hypersensitivity pulmonary disease that affects primarily infants; and in a patient with Lane-Hamilton syndrome, a rare combination of idiopathic pulmonary hemosiderosis and celiac disease.[108–112] Siderophages were also found in respiratory samples collected from patients diagnosed with rheumatoid arthritis and juvenile dermatomyositis.[113,114]

Studies suggest an elevation of siderophages in autopsy samples from victims of sudden infant death syndrome (SIDS), unexpected infant death and/or suffocation, and chronic physical child abuse.[115–121] Finally, macrophage activation syndrome, fever without pulmonary infiltrates among the immunocompromised (in AIDS and non-AIDS patients), and mucolipidosis have been associated with siderophages.[5,122,123]

V. PPB STAIN AND IRON HOMEOSTAIS

In vivo staining of lung tissue, BAL, and sputum with PPB can possibly be the result of exposure to either excess iron (e.g., metal included in hemoglobin) or compounds or substances that function to complex iron (e.g., particles, fibers, and phenolic compounds). An *in vitro* production of iron-laden macrophages is straightforwardly accomplished by exposing human alveolar macrophages collected from healthy subjects to either ferric citrate or ferric ammonium citrate at concentrations as low as 20–100 μM (exposures to iron chlorides and sulfates do not work) (Fig. 4A–D). The time required for the *in vitro* formation of siderophages with these concentrations of iron can be as short as 2 hours. In addition, cell types other than macrophages can be stained with PPB using *in vitro* exposures, introducing the question of the relevance of findings from this approach of formation to understanding of *in vivo* staining. Regarding *in vivo* cell staining with PPB resulting from an excess of iron, concentrations of available metal in any cell likely approach only 1–5 μM.[124] However, macrophages are extremely adept at both metal uptake and its storage in ferritin.[125] Therefore, to maintain efficient function and maximal utility in the lower respiratory tract, lung macrophages export excess metal and this can include ferritin release.[126,127] Specifically addressing the potential of hemorrhage leading to PPB stain, a majority of the iron required to support heme biosynthesis in the human originates from: senescent

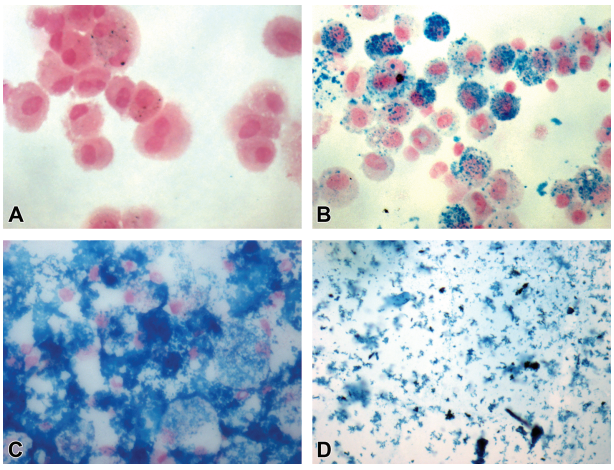

FIG. 4: *In vitro* production of siderophages. Alveolar macrophages collected by lavage from healthy subjects show few hemosiderin-laden macrophages (1–3%) (A). Following *in vitro* exposure of the macrophages to 100 μM ferric ammonium citrate, there is an increased number of cells that stain with PPB at 24 hours (B). The incubation of alveolar macrophages with 100 μM ferric chloride and ferric and ferrous sulfates does not produce the classic iron-laden macrophage but rather oxy-hydroxy precipitates of iron with staining positive for the metal between the cells outlining them (C). Acellular incubation of 100 μM iron (III) sulfate for 24 hours with cytocentrifuge reveals a positive stain for oxy-hydroxides (D). Magnification for A to D approximates 400×.

erythrocytes engulfed by macrophages, breakdown of the heme by heme oxygenase, some portion of the metal being bound to ferritin, and release of metal by the macrophage for ultimate use by the erythroblast.[128] After erythrophagocytosis, macrophages release iron and ferritin that is then endocytosed and utilized by other cells.[129,130] Iron released through activity of heme oxygenase can impact metal homeostasis including the serum concentrations of ferritin and iron possibly reflecting the release of both by macrophages.[131] As a result of their participation in the recycling of iron from effete erythrocytes, it might be anticipated that macrophages would be infrequently overwhelmed by the challenge of bleeding and this would rarely contribute to the formation of siderophages. Incubation of alveolar macrophages with enormous numbers of normal, undamaged erythrocytes does not produce siderophages. However, despite many pathways for iron uptake, storage, and release, lung macrophages appear to accumulate significant concentrations of iron following excess iron after hemorrhage. Accordingly, it can be assumed that an exposure of the lung macrophage to erythrocytes can overwhelm the capacity to store and export the metal, be associated with production of hemosiderin, and stain with PPB.

Staining of intracellular and extracellular structures and cells for PPB more frequently results from exposure of the lung to compounds or substances that function to complex iron. Intracellularly, the compound or substance is frequently located in a phagolysosome. Inorganic and carbonaceous particles and fibers introduce a solid-liquid interface into a cell that includes oxygen-containing functional groups at the surface (e.g., silanol groups on silica and silicates). Such an open network of negatively charged functional groups on a particle and fiber surface presents spaces large enough to accommodate adsorbed metal cations. Iron is kinetically preferred among the cellular cations available for complexation by an inorganic particle or fiber surface.[132] Carbonaceous particles (e.g., cigarette smoke and ambient air pollution) frequently include humic-like substances (HULIS) in cigarette smoke particles and emission and ambient air particles.[133,134] Oxygen-containing functional groups in HULIS, including carboxylate, phenolate, keto, keto-enol, and carbonyl groups, favor the formation of stable complexes with metals and the sorption ability of iron is greatest among all of them.[135–139] Other organic particles and fibers include alternative compounds or substances that have a comparable capacity to complex iron (e.g., cotton is comprised of cellulose, which is recognized to complex iron, accounting for the formation of "byssinotic bodies").[140,141]

The source of the metal that accumulates during the formation of a ferruginous body and a siderophage is unlikely to be from the exposure since both develop after exposures that do not have iron, e.g., chrysotile asbestos and wood smoke particle.[142] Intracellular sources for iron that accumulate onto the particle and fiber surface include that complexed with ATP, ADP, GTP, citrate, DNA, and free amino acids.[143] The formation of a ferruginous body occurs only in intracellular locations because the

complexation of extracellular sources of iron (e.g., transferrin, lactoferrin, and ferritin) by a particle or fiber is not possible due to the extremely strong binding of the metal with these proteins.

The macrophage responds to loss of its essential iron by attempting to acquire metal necessary for its continued survival (Fig. 5). Reductants available in the lung (e.g., superoxide) will reduce complexed Fe^{3+} to Fe^{2+} and displace it from the compound or substance that functions to complex metal (e.g., the particle and fiber surface). This is comparable to the reduction of iron complexed to transferrin by ferrireductases generating superoxide at the membrane of an endocytosed vacuole (e.g., STEAP1). After reduction and displacement from the surface, the metal is transported to the cell through the activity of an importer (e.g., divalent metal transporter 1, DMT1). The metal will interact with an iron responsive protein/iron responsive element to affect elevations in iron importers (e.g., transferrin receptors and DMT1), exporters (e.g., ferroportin), and storage proteins (e.g., apoferritin).[144] The iron can be stored in ferritin after its re-oxidation to Fe^{3+} in an O_2-containing environment. However, the compound or substance that functions to complex metal (e.g., the particle and fiber surface) retains this capacity and will again initiate the cycle. This series of reactions occurs close to the compound or substance that functions to complex metal (e.g., the particle and fiber surface). The recurrent complexation with the subsequent reduction and oxidation during the contest for iron between the exposure and the macrophage will result in an accrual of ferritin and, with oxidant- and protease mediated damage, hemosiderin. Elevations in the iron-storage proteins will initially be observed in the immediately proximity of the compound or substance that functions to complex metal (e.g., the particle and fiber surface), but these are predicted to eventually involve the entire cell, supporting the observed relationship between ferruginous bodies and siderophages.

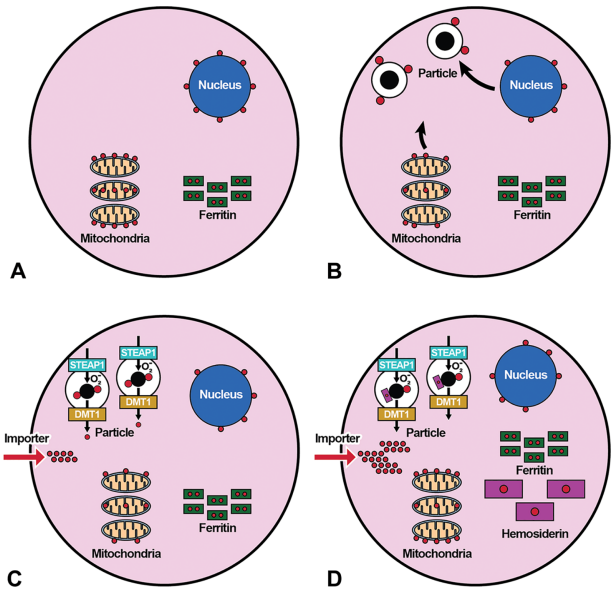

FIG. 5: Schematic demonstrating iron homeostasis in the cell (A) and its disruption by a particle, fiber, or compound or substance with functional groups (in a phagolysosome) that can complex cell metal (iron is represented by red balls) (B). The cell will compete for its own iron now complexed to the compound or substance (here a particle surface) using ferrireduction (e.g., STEAP1) by superoxide followed by uptake of the metal (e.g., DMT1) (C). The loss of iron by the cell organelles to the particle surface imparts an iron deficiency and the metal import from the extracellular environment will be increased. Total concentrations of iron will increase, allowing continued cell survival, and there will be elevations in ferritin and hemosiderin, with the latter resulting from exposure to oxidants and proteases (D).

The inhomogeneity of the ferruginous body reflects a preferential deposition of metal (and protein) at specific sites along the particle and fiber surface. In a particle and fiber, polymeric units (e.g., silicon dioxide) may alternate with the hydroxides/oxides of larger cations (e.g., SiO and MgO alternating in chrysotile). The cleavage, parting, and fracture of these minerals will result in fibers with significant variability in the placement of functional groups at the surface (e.g., $Si\text{-}O^-$ alternating with $Mg\text{-}O^-$ in chrysotile). Such functional groups will deprotonate at different pH values.[145] Therefore, iron will preferentially accumulate at those regions of surface populated by the more acidic functional groups (e.g., the silanol groups in a silicate fiber). Quaternary ammonium groups of proteins, including both ferritin and hemosiderin, may also be adsorbed onto those portions of the particle and fiber surface that are more negatively charged. The inhomogeneous

appearance of some ferruginous bodies (e.g., asbestos bodies) reflects this disparate placement of functional groups on the surface of a particle and fiber with an uneven adsorption of both metals and proteins.

Following exposure to compounds or substances with the capacity to complex iron (including particles and fibers), sequestration of its requisite metal affects an increased iron import by the host cell (Fig. 6). Macrophages exposed to particles and fibers respond to the immediate loss of its metal to the surface functional groups with attempts to acquire more metal.[127,146] Increased import of iron exposed to particles and fibers reflects a functional metal deficiency in these cells following complexation of the host metal by the surface functional groups. Cell iron importers are affected by such a functional iron deficiency and contribute to an increased metal uptake; an elevated expression of such an importer follows particle and fiber exposure.[147–149] Superoxide production by the cell exposed to particles and fibers increases ferrireduction that is required prior to metal import.[148] Cell ferritin, as well as hemosiderin, concentrations also increases following such exposure.[126,146,149] Subsequently, stored metal concentrations increase and siderophages develop.[40] Despite this accumulation of metal and staining for PPB, that iron available for cell function is predicted to be decreased as a significant portion of the metal remains sequestered by the particle and fiber.

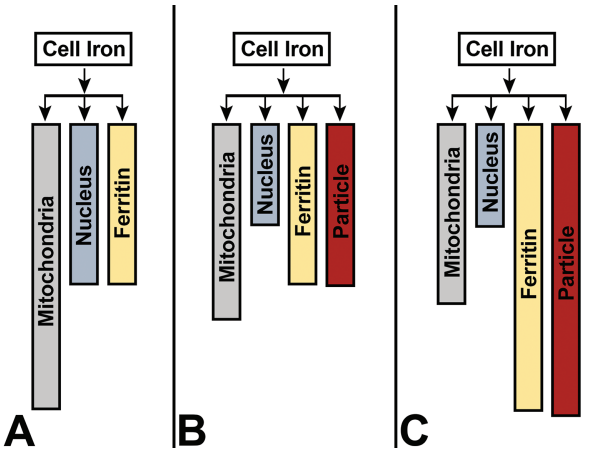

FIG. 6: Schematic depicting changes in provisional iron pools in the cell with exposure to a compound or substance that disrupts iron homeostasis by complexing metal (e.g., particles and fibers). Iron pools in the cell (A) are altered with decreased iron concentrations associated with mitochondria and nuclei, whereas metal is complexed by surface functional groups on the particle (B). Following the response of greater ferrireduction and augmented expression of metal importers to move iron into the cell, iron pools in the cell increase, allowing continued function and survival of the cell. However, a greater part of the metal is associated with the particle, effecting a continued functional iron deficiency in the cell (C).

Teleologically, changes in iron homeostasis following exposures to compounds or substances that disrupt cell iron homeostasis (e.g., particles and fibers) are likely to have evolved originally as a response to infectious agents. Microbes demonstrate an absolute requirement to utilize iron from the host cell to support their duplication and survival. Subsequently, the capacity to acquire iron from the host frequently determines the virulence of bacteria.[150] Exposure to microbes is associated with an increase in cell iron concentration.[151] A functional metal deficiency follows exposure of alveolar macrophages to microbials with an elevated expression of importers similar to the host response to particles.[152] In addition, microbials increase ferritin expression in exposed cells.[152,153]

VI. CONCLUSIONS

In lung tissue, BAL, and sputum, the presence of structures and macrophages that stain with PPB (i.e., ferruginous bodies and siderophages) is associated with numerous exposures and diseases. Many of the exposures function to complex cell iron and impact an accumulation of metal that is observed on PPB stain. Numerous lung diseases and extrapulmonary diseases demonstrate increased numbers of siderophages but some of these can be associated with exposures to compounds or substances with capacity to complex iron and to accordingly disrupt iron homeostasis (e.g., cigarette smoke particle). Despite increased total iron concentrations, a deficiency of the metal may exist in the cell since significant amounts of iron are sequestered by the compound or substance after exposure. Novel applications for this

stain continue to evolve with attempts to employ it as an indicator of iron homeostasis.[154–158]

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