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Role of iron in the pathogenesis of respiratory disease

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Abbreviations: ARDS: Acute respiratory distress syndrome; TRALI: Transfusion-related acute lung injury; IPF: Idiopathic pulmonary fibrosis; CF: Cystic fibrosis; COPD: Chronic obstructive pulmonary disease; PAP: Pulmonary alveolar proteinosis; BAL: Bronchoalveolar lavage; DCYTB: Duodenal cytochrome b; DMT1: Divalent metal transporter 1; FPN: Ferroportin; LF: Lactoferrin; LFR: Lactoferrin receptor; slc11a1: solute carrier family 11 member 1; RBC: Red blood cells; TF: Transferrin; TFR: Transferrin receptor; IFN- γ : interferon- γ ; TNF- α : tumor necrosis factor- α ; TFBP: TF binding protein; non-small-cell: NSC; ferritin-bound iron: FBI; hypoxia inducible factor-1 α : HIF-1 α ;

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Abstract

Iron is essential for many biological processes, however, too much or too little iron can result in a wide variety of pathological consequences, depending on the organ system, tissue or cell type affected. In order to reduce pathogenesis, iron levels are tightly controlled in throughout the body by regulatory systems that control iron absorption, systemic transport and cellular uptake and storage. Altered iron levels and/or dysregulated homeostasis have been associated with several lung diseases, including chronic obstructive pulmonary disease, lung cancer, cystic fibrosis, idiopathic pulmonary fibrosis and asthma. However, the mechanisms that underpin these associations and whether iron plays a key role in the pathogenesis of lung disease are yet to be fully elucidated. Furthermore, in order to survive and replicate, pathogenic micro-organisms have evolved strategies to source host iron, including freeing iron from cells and proteins that store and transport iron. To counter these microbial strategies, mammals have evolved immune-mediated defence mechanisms that reduce iron availability to pathogens. This interplay between iron, infection and immunity has important ramifications for the pathogenesis and management of human respiratory infections and diseases. An increased understanding of the role that iron plays in the pathogenesis of lung disease and respiratory infections may help inform novel therapeutic strategies. Here we review the clinical and experimental evidence that highlights the potential importance of iron in respiratory diseases and infections.

1. Introduction

Iron is a key nutritional trace element that plays a role in many different biological processes, including DNA and RNA synthesis, oxygen transport, cellular respiration, the activity of numerous enzymes, immune function and metabolism (Beard, 2001; Rouault and Tong, 2008). Too little iron can have deleterious consequences that result from deficits in key biological processes. However, too much iron can also have harmful effects, in particular through the generation of reactive oxygen species (ROS), which induce oxidative stress, lipid peroxidation, and DNA damage. This results in genomic instability and DNA repair defects that affect cell viability and promote programmed cell death (Paul and Lill, 2015; Zhang, 2014).

In humans, 60-70% of iron is contained within haemoglobin in red blood cells (RBC) (Geissler and Singh, 2011). Since various organs, including bone marrow, spleen and liver, are involved in RBC production and clearance, these organs function as key compartments for iron storage and recycling (Andrews and Schmidt, 2007; Ganz and Nemeth, 2006). A further 10% of body iron is found in muscle myoglobin and mitochondrial cytochromes are also rich sources (Geissler and Singh, 2011). Outside these sites, most iron in the body is stored intracellularly in ferritin, whilst most extracellular iron is associated with high-affinity, iron-binding proteins such as transferrin (TF) (Andrews and Schmidt, 2007).

Under healthy conditions, finely tuned iron regulatory mechanisms maintain systemic and cellular iron homeostasis and prevent toxicity by balancing the body's iron demand through absorption, recycling of haem-iron from RBCs and storage and mobilization processes (Andrews and Schmidt, 2007; Meyron-Holtz et al., 2014; Philpott and Ryu, 2014) (**Fig 1**). These processes are tightly controlled and involve a number of iron-binding and -storing proteins, iron-sensing receptors and transporting factors and both intracellular and intercellular signalling molecules (Andrews and Schmidt, 2007; Cassat and Skaar, 2013; Meyron-Holtz et al., 2014; Nairz et al., 2014; Philpott and Ryu, 2014; Skaar, 2010; Soares and Weiss, 2015; Weinberg, 2009) (**Fig 1**).

Dysregulation of iron homeostasis has been associated with numerous diseases including haemochromatosis and anaemia as well as several cancers, and cardiovascular, neurodegenerative and respiratory diseases (Gozzelino and Arosio, 2016). Respiratory diseases account for up to 1 in 6 deaths globally and are a major socio-economic burden (Foster et al., 2013; Hansbro et al., 2013; Keely et al., 2012; Kim et al., 2015; WHO, 2011). There is strong evidence for dysregulated iron homeostasis in several major respiratory diseases, including chronic obstructive pulmonary disease (COPD), lung cancer, idiopathic

pulmonary fibrosis (IPF), cystic fibrosis (CF), asthma and acute respiratory distress syndrome (ARDS). Respiratory infections are a leading cause of death worldwide and are a major contributor to the pathogenesis of lung disease (Foster et al., 2013; Hansbro et al., 2013; Keely et al., 2012; Kim et al., 2015; WHO, 2011). Humans have a range of host defences that protect against microbial invasion as well as limit the growth and replication of pathogens within the respiratory tract. These include epithelial and other barriers that prevent pathogen access to host tissues as well as innate and adaptive immune responses (Hallstrand et al., 2014). Since iron is also required for microbial biological processes, one of the key defence strategies in humans to protect against infection is the limitation of iron supply to pathogens (Cassat and Skaar, 2013; Nairz et al., 2014). Importantly, many clinically important respiratory pathogens have developed strategies that enable them to efficiently source iron from the host.

Here we describe how iron homeostasis is regulated in the human body, outline the current understanding of clinical and experimental evidence that supports a link between dysregulated iron and/or iron regulatory molecule responses and lung disease and highlight the importance of the interplay that exists between iron, infection and immunity in the context of respiratory infection.

2. Overview of iron homeostasis

Iron levels are tightly controlled in throughout the body. Iron in the diet is found in both non-haem and haem forms, with most of the non-haem iron present as ferric ions (Fe^{3+}) (Andrews and Schmidt, 2007; Ganz and Nemeth, 2006; Geissler and Singh, 2011). Whole-body iron content is maintained within a relatively narrow range through co-ordinated mechanisms that control iron uptake, storage, utilisation and excretion (Andrews and Schmidt, 2007; Hentze et al., 2004). During digestion, Fe^{3+} is converted to Fe^{2+} by membrane metalloreductase proteins or by other reducing agents, such as ascorbic acid, in the gastrointestinal tract. This enables transport of iron from the lumen into enterocytes through the divalent metal transporter (DMT)1 protein (Andrews and Schmidt, 2007; Lambe et al., 2009; McKie et al., 2001) (**Fig 1**). Following DMT1-mediated absorption, a proportion of iron is stored in enterocytes bound to ferritin, whilst some is released into the circulation by the iron export protein, ferroportin (FPN) (Andrews and Schmidt, 2007; Geissler and Singh, 2011; Wessling-Resnick, 2006). In the blood, iron generally binds to TF and is transported to different tissues where it is utilised for numerous iron-dependent functions (Andrews and Schmidt, 2007; Geissler and Singh, 2011). Iron that is loaded into TF (holo-TF) interacts with

transferrin receptor (TFR)1 on the cell membrane, resulting in internalisation of the complex *via* endocytosis (Andrews and Schmidt, 2007; Hentze et al., 2004). Iron is then released from TF into the endosome following Na⁺-H⁺-ATPase-mediated endosome acidification before being released into the cytoplasm *via* DMT1 (Andrews and Schmidt, 2007; Gunshin et al., 1997; Hentze et al., 2004). TF and TFR1 are recycled back to the cell surface with TF released into the extracellular environment and TFR1 retained in the membrane. The iron released into the cytoplasm is either utilised, stored as ferritin or incorporated into various iron-containing molecules, including haemoglobin, myoglobin or cytochromes, depending on the cell type and requirement (MacKenzie et al., 2008; Philpott and Ryu, 2014). Ferritin functions as a major intracellular storage molecule for iron, limiting excessive free iron that could otherwise generate ROS (Philpott and Ryu, 2014; Zhang, 2014).

Intracellular iron levels are regulated by iron regulatory proteins (IRP)1 and 2, which can bind to iron-responsive elements (IRE) present in the 3' and 5' untranslated regions (UTR) of mRNA transcripts for iron homeostasis regulatory factors, including ferritin, FPN and TFR1, as well as factors not directly involved in iron regulation (Erlitzki et al., 2002; Kato et al., 2007). When intracellular iron concentrations are low, the binding activity of IRPs is high, which results in the stabilisation of transcripts containing an IRE in the 3' UTR (e.g. TFR1), and repression of translation of transcripts containing an IRE in the 5' UTR (e.g. ferritin) (Muckenthaler et al., 2008). The net effect is increased TFR1 and decreased ferritin expression, supporting the restoration of intracellular iron to healthy physiological levels. Conversely, when labile intracellular iron concentrations are high, the binding of IRPs to IREs is decreased, resulting in reductions in TFR1 and increased ferritin (Muckenthaler et al., 2008).

Once iron is absorbed, there are no specific active physiological mechanisms that exist to excrete iron from the body, although small amounts of iron are lost in urine, sweat and shedding of epithelial cells. Instead the maintenance of iron levels is controlled by homeostatic mechanisms that regulate absorption, storage and re-utilisation rather than through active excretion (Anderson, 2007; Geissler and Singh, 2011).

2.1. Key role of hepcidin in iron homeostasis

Hepcidin is a cysteine-rich 25 amino acid cationic peptide predominantly expressed in the liver that plays a crucial role in iron homeostasis (Andrews and Schmidt, 2007; Krause et al., 2000; Nicolas et al., 2002a) (**Fig 1**). Hepcidin regulates intra- and extracellular iron levels by reducing the efflux of iron from hepatocytes and tissue macrophages, which are the cells

responsible for the storage of iron, as well as by decreasing the rate of iron transport from enterocytes into the bloodstream, which effectively reduces dietary iron absorption (Andrews and Schmidt, 2007). Hepcidin accomplishes this by binding to the cellular transmembrane iron exporter, FPN leading to its endocytosis and proteolysis in hepatocytes, macrophages and enterocytes, which results in decreased FPN-mediated iron transport into extracellular fluids and increased cellular iron retention (Nemeth et al., 2004).

Hepcidin expression is increased by high iron levels and inflammation (Nicolas et al., 2002b; Pigeon et al., 2001), and decreased by iron deficiency, ineffective erythropoiesis and hypoxia (Nicolas et al., 2002b; Weinstein et al., 2002). Increased iron levels in the plasma increase hepcidin expression through a number of pathways, which in turn reduces iron absorption through the gut and iron release from cells into the circulation (Knutson et al., 2005; Laftah et al., 2004; Lin et al., 2007; Pigeon et al., 2001; Rivera et al., 2005; Yamaji et al., 2004) (**Fig 1**). Under normal physiological conditions, hepcidin expression is regulated in response to iron through the bone morphogenetic protein (BMP)/SMAD pathway and the haemochromatosis protein (HFE)/TFR1/TFR2 signalling pathway. Elevated liver iron increases the expression of BMPs, notably BMP6, which bind to type I and II cell BMP receptors and the BMP co-receptor, haemojuvelin (HJV), on the cell surface (Core et al., 2014; Pantopoulos et al., 2012; Parrow and Fleming, 2014). This results in the phosphorylation of SMADs 1, 5 and 8, and subsequent interaction with SMAD4 (Core et al., 2014; Pantopoulos et al., 2012; Parrow and Fleming, 2014). The SMAD complex is then transported into the cell nucleus where it binds to BMP-responsive elements (BMPRE) within the hepcidin promoter, resulting in the increased expression of hepcidin (Core et al., 2014; Pantopoulos et al., 2012; Parrow and Fleming, 2014). The BMP signalling pathway is also adjusted by two sensors of holo-TF (i.e. iron-saturated TF), TFR1 and 2, and their binding partner, the MHC class I-like transmembrane protein, HFE. Increasing concentrations of holo-TF alters the interaction of HFE from TFR1 to TFR2, which stimulates the stabilisation of the TFR2 protein (Schmidt et al., 2008). TF:TFR2 binding is proposed to promote hepcidin expression through the modification of BMP/SMAD and ERK/MAPK signalling pathways (Casanovas et al., 2009). Defects in HFE and TFR2 lead to decreased hepcidin expression, which results in excessive iron loading, whilst restoration of HFE and TFR2 expression in HFE deficient and TFR2 mutant mice restores hepcidin expression and reverses excessive iron loading (Fleming et al., 2002; Gao et al., 2010; Levy et al., 1999; Schmidt et al., 2008; Zhou et al., 1998). Defects in the HFE and/or TFR2 genes are responsible for the pathogenesis of excessive iron loading in most patients with hereditary haemochromatosis,

with mutations in HFE being most commonly associated with the disease and TFR2 mutations rare (Bridle et al., 2003; Gehrke et al., 2003; Powell et al., 2016).

3. Inter-relationship between iron, infection and immunity

3.1 Iron and infection

Iron is essential for the optimal growth and replication of the majority of microorganisms, including human pathogens (Cassat and Skaar, 2013; Parrow et al., 2013; Payne, 1993). Invading bacterial pathogens use a variety of strategies to source iron from host tissues. Whether the pathogens are extracellular or intracellular microbes affects the strategies they use to source iron. In addition, microbial iron acquisition strategies depend on the different forms of iron that the microbes target, such as labile iron or iron associated with haem or TF. Iron sourcing mechanisms include haem acquisition systems, production of siderophores and, utilisation of ferric or ferrous iron transporters or TF or lactoferrin (LF) binding proteins (Cassat and Skaar, 2013; Parrow et al., 2013).

Clinical observations have shown that excess iron in haemochromatosis patients increases susceptibility to various pathogens, including *Escherichia coli*, *Vibrio vulnificus*, *Vibrio cholera*, *Klebsiella* species, *Listeria monocytogenes*, *Shigella* species, Hepatitis B and C, cytomegalovirus, parvovirus, and HIV (Bullen et al., 1991; Cappellini MD, 2008; Christopher, 1985; Fernandez et al., 2000; Hopfner et al., 2001; Khan et al., 2007; Parrow et al., 2013; Wanachiwanawin, 2000). A study of 1,455 haemodialysis patients showed a significant increase of bacteraemia incidence in those with high serum ferritin levels (Kessler et al., 1993). Another study of 607 haemodialysis patients also revealed that high serum ferritin is an independent risk factor for bacterial infection in these patients (Hoen et al., 1995). *Salmonella* infections have been found to be increased in patients with haemolytic disorders such as malaria and sickle cell disease (Jurado, 1997). Moreover, patients with beta-thalassaemia, a disease that often requires repeated blood transfusions that result in iron overloading, are susceptible to infection with normally avirulent pathogens, such as *Yersinia enterocolitica* (Green, 1992; Wanachiwanawin, 2000).

3.2 Innate immune responses limit iron availability during infection

Since iron is essential for both humans and clinically important pathogens, the outcome of competition between host defensive iron sequestration strategies and counter-strategies of pathogens for sourcing iron plays an important role in determining the fate of an infection (Soares and Weiss, 2015; Sutak et al., 2008; Traore and Meyer, 2007).

One of the key defence strategies that protects against infection by extracellular microbes is the limitation of labile iron to extremely low levels in extracellular fluids in the body. Most iron is stored intracellularly in complexes with proteins such as haem and ferritin, whilst extracellular iron is strongly bound to TF. However, TF-bound iron is also a potential source of iron for extracellular microbes (Hunter et al., 1984; Schryvers and Morris, 1988; Vogel et al., 1997a). Apart from TF, a series of iron-binding proteins, including LF, sequester iron. LF is a powerful iron chelator found in various mucosal secretions that limits iron availability to pathogens (Baveye et al., 1999). LF is released by epithelial cells as well as by neutrophils that have infiltrated to infection sites (Ward et al., 2002). Importantly, studies show that administration of LF to mice infected with bacteria bacterial growth (Drago-Serrano et al., 2010; Hwang et al., 2014; Velliyagounder et al., 2015; Zagulski T, 1989). These findings suggest that LF may protect against infection by limiting iron availability, although, LF also has other immunomodulatory and antimicrobial activities that may contribute to its protective effects (Actor et al., 2009; Drago-Serrano et al., 2010; Fischer et al., 2006; Hwang et al., 2014; Ward et al., 2002). In addition to LF, lipocalin (LCN)2 (also known as neutrophil gelatinase-associated LCN [NGAL] and siderocalin) is released from neutrophils and epithelial cells in response to infection and inflammatory stimuli. LCN2 binds pathogen-specific salicylate- and catecholate-type iron sourcing molecules, which hinders the ability of pathogens that utilise these types of siderophores to source iron (Chan et al., 2009; Cowland et al., 2003; Flo et al., 2004; Goetz et al., 2002; Holmes et al., 2005). Like LF, LCN2 also appears to have immunomodulatory effects.

As discussed previously, hepcidin regulates iron levels by inhibiting FPN-mediated iron efflux from cells. Importantly, hepcidin plays a key role in innate immune-mediated iron limitation (Kemna et al., 2005). During infection and inflammatory responses, hepcidin production is up-regulated through activation of STAT3 signalling by IL-6 (Wrighting and Andrews, 2006). Other cytokines, such as IL-1 α , IL-1 β and IL-22, also stimulate intracellular cytokine signalling pathways that increase hepcidin expression (Armitage et al., 2011; Lee et al., 2005; Smith et al., 2013a). Macrophages and neutrophils produce hepcidin, regulating iron bioavailability at the site of infection (Peyssonnaud et al., 2006). Hepcidin also affects FPN in an autocrine fashion to regulate intracellular iron content in monocytes during inflammatory responses (Theurl et al., 2008). This coordinated program depletes extracellular iron to limit its availability to pathogens. Interestingly, FPN expression can also be down-regulated in monocytes by inflammatory cytokines through hepcidin-independent mechanisms (Ludwiczek et al., 2003).

Immune responses limit iron availability to intracellular pathogens by altering the transcript expression and/or translation of iron-regulatory molecules, including ferritin, TFR1, FPN solute carrier family 11 member 1 (slc11a1, formerly NRAMP1), DMT1, haem oxygenase (HOX)1 and LCN2, in infected monocytes and macrophages (Byrd and Horwitz, 1993; Johnson et al., 2010; Nairz et al., 2008; Nairz et al., 2007; Olakanmi et al., 2002). For example, studies show that IFN- γ decreases the expression of ferritin and TFR1 in human monocytes (Byrd and Horwitz, 1993). IFN- γ can also cause decrease iron uptake through TFR1, and increase iron export by FPN in infected murine macrophages (Nairz et al., 2008; Nairz et al., 2007). This reduction in TFR1 expression and increase in FPN limits the cell's ability to engulf holo-TF and increases iron efflux, reducing intracellular iron availability to the pathogen and inhibiting its replication. The transporter slc11a1/NRAMP1 is an integral membrane protein found in the endosomes of macrophages that is responsible for exporting iron and other divalent metals (Mn²⁺, Zn²⁺) out of the infected endosome, limiting iron bioavailability. It has been shown to play an important role in protecting against several species of intracellular pathogens (Caron et al., 2002; Gruenheid et al., 1997; Jabado et al., 2000; Vidal et al., 1995a; Vidal et al., 1995b; Zwilling et al., 1999). Interestingly, This may occur through the restriction of the expression of IL-10 and increased expression of inducible nitric oxide synthase (iNOS) by macrophages, which dramatically enhances the ability of macrophages to restrict microbial growth (Fritsche et al., 2008).

4. Regulation of iron levels in the lung

Iron-regulatory molecules, including ferritin, TF, LF, TFR1 and 2, LFR, FPN, DMT1, hepcidin, slc11a1/NRAMP1, LCN2 and duodenal cytochrome c (DCYTB) are produced by different cell types within the lung, including epithelial cells and macrophages (Basaraba et al., 2008; Cowland et al., 2003; Frazier et al., 2011; Ghio et al., 1999; Ghio et al., 1998; Ghio et al., 2006; Giorgi et al., 2015; Nguyen et al., 2006; Upton et al., 2003; Wang et al., 2002; Wesselius et al., 1994; Wu et al., 2011; Yang et al., 2002) (**Fig 2**). As in other tissues, there is evidence to support roles for these iron-regulatory molecules in the lung in regulating the transport of iron between the blood and tissue, maintaining intracellular and extracellular iron levels required for biological function and ensuring that iron is stored in a manner that protects the lungs from iron-induced oxidative stress and infection (Basaraba et al., 2008; Cowland et al., 2003; Frazier et al., 2011; Ghio et al., 1999; Ghio et al., 1998; Ghio et al., 2006; Giorgi et al., 2015; Nguyen et al., 2006; Upton et al., 2003; Wang et al., 2002; Wesselius et al., 1994; Wu et al., 2011; Yang et al., 2002).

Import of non-TF-bound iron into lung epithelial cells is mediated by DMT1 and DCYTB (Ghio et al., 2006; Giorgi et al., 2015). Decreased systemic iron as a result of repeated bleeding in a mouse model of iron deficiency has been shown to have no effect on lung iron levels but increases DMT1, HFE and TFR1 expression in the lung as well as localisation of DMT1, HFE and FPN to apical surface of airways epithelial cells (Giorgi et al., 2015). In contrast, increased systemic iron levels as a result of intraperitoneal iron saccharate injections, has been shown to increase lung iron levels which corresponds with an increase in FPN expression in the lung (Giorgi et al., 2015). Interestingly, increased lung iron associated with increased systemic iron also resulted in increased localisation of DMT1 to the apical surface of airway epithelial cells, which supports an important role for DMT1 airway epithelial cell surface expression in maintaining lung iron homeostasis (Giorgi et al., 2015).

In the lung, iron is stored intracellularly in ferritin, limiting its ability to form free radicals. It has been shown that elevated production of ferritin in lung epithelial cells and macrophages occurs rapidly following iron exposure (Ghio et al., 1998). Furthermore, increased iron levels in the lung following systemic iron loading, results in the localisation of ferritin near to the apical surface of airways epithelial cells and increased iron accumulation in macrophages (Giorgi et al., 2015).

Macrophages play a principal role in maintaining iron homeostasis through the sequestration of haem-iron from senescent RBCs for storage and recycling, the uptake and storage of TF-bound iron and, in the lung, the clearance of inhaled iron-containing particulate matter (Gammella et al., 2014; Ganz, 2012) (**Fig 1 & 2**). As such, the handling and storage by macrophages of relatively large amounts of iron from different sources plays an important role in protecting the lung from iron-induced oxidative stress and sequestering iron from potential invading pathogens (Corhay et al., 1992; Fritsche et al., 2007; Mulero et al., 2002; Nairz et al., 2009; Soe-Lin et al., 2009; Turi et al., 2004; Zwilling et al., 1999) (**Fig 1 & 2**). Neutrophils have also been shown to be involved in iron homeostasis by releasing several iron regulatory molecules, including LF and LCN2, which also help sequester free iron and protect the lung (Ghio et al., 2006) (**Fig 2**).

5. Altered iron levels and iron regulatory molecule responses in lung disease

5.1 Cigarette smoking, COPD/emphysema and lung cancer

Cigarette smoke contains iron particulates and toxic substances that can induce intracellular iron accumulation and alter iron homeostasis in the lung and systemically (Ghio et al., 2008a). Iron and ferritin levels are increased in the lungs of smokers and macrophages

in the lower respiratory tract have increased iron loading following smoke exposure (Nelson et al., 1996; Thompson et al., 1991). Furthermore, cigarette smoke increases iron accumulation in the airways, which results in elevated oxidative stress and release of IL-8 (Ghio et al., 2008a) and excess iron in the alveoli has been shown to contribute to increased alveolar macrophage-derived IL-1 β production (O'Brien-Ladner et al., 2000), suggesting a potential relationship between iron accumulation and inflammation in the progression of cigarette smoking-associated diseases.

COPD is a complex progressive condition characterized by chronic airway inflammation that is associated with airway remodelling, alveolar destruction (i.e. emphysema) and persistent airflow obstruction and is predicted to be the 3rd leading cause of death world-wide by 2030 (Beckett et al., 2013; Budden et al., 2016; Fricker et al., 2014; Lozano et al., 2012; Rabe et al., 2007). Whilst environmental factors including fossil fuel smoke exposure and diet and certain genetic risk factors have been shown to increase susceptibility to disease, cigarette smoking is the single greatest risk factor for developing COPD (Beckett et al., 2013; Chappell et al., 2011; Fricker et al., 2014; Hanson et al., 2014; Huang et al., 2013; WHO, March 2015). Altered macrophage iron sequestration and levels of iron-binding proteins including TF, ferritin, haptoglobin and LCN2 as well as hepcidin have been detected in both respiratory fluids and serum of patients with COPD and emphysema (Eagan et al., 2010; Engstrom et al., 2009; Nelson et al., 1996; Philippot et al., 2014; Tandara et al., 2015). Significantly, alveolar macrophages have been shown to have increased iron loading in COPD with the quantity of iron deposits and percentage of iron-positive macrophages associated with disease severity (Philippot et al., 2014). Genome-wide association studies have shown relationships between COPD susceptibility and some single-nucleotide polymorphisms (SNPs) in several genes associated with iron regulation (Chappell et al., 2011; Pillai et al., 2009). Notably, IRP2 has a strong association with susceptibility to COPD (Chappell et al., 2011; DeMeo et al., 2009). The expression of IRP2 has been shown to be significantly increased in the lung tissues of COPD patients compared to controls, with an increased trend of upper lobe emphysema shown to be associated with five IRP2 SNPs (Chappell et al., 2011; DeMeo et al., 2009). Interestingly, the effects of IRP2 SNPs on COPD may be independent of smoking (Zhou et al., 2012). IRP2 was also found to be significantly associated with emphysema in males with α 1-antitrypsin deficiency, which is associated with a strong increase in susceptibility to COPD independent of smoke exposure (Kim et al., 2012). Significantly, an elegant study recently published by Cloonan *et al.*, provides valuable

mechanistic insights into the relationship between smoking, dysregulated iron homeostasis and oxidative stress-induced mitochondrial dysfunction in COPD pathogenesis (Cloonan et al., 2016). The authors show that IRP2 is an important COPD susceptibility gene, that IRP2 responses are abnormally increased in patients with COPD and that IRP2 deficient mice are protected against the effects of chronic cigarette smoke exposure (Cloonan et al., 2016; DeMeo et al., 2009). The authors then went on to demonstrate that increased IRP2 results in increased iron accumulation in the mitochondria and that this leads to cytochrome c oxidase-dependent mitochondrial dysfunction that is responsible for the pathogenesis of key features of disease. Furthermore, they showed that mice administered with a mitochondrial iron chelator or fed a low-iron diet were protected (Cloonan et al., 2016). These findings highlight the importance of dysregulated iron homeostasis in the pathogenesis of COPD and also demonstrate the potential for iron-targeted therapeutic strategies for the management/treatment of disease.

Lung cancer is the leading cause of cancer-related morbidity and mortality worldwide. Approximately 80-90% of lung cancer is associated with cigarette smoking (Alberg et al., 2007; Thun et al., 2008; WHO, 2014). Increased levels of ferritin have been found in the serum, bronchoalveolar lavage (BAL) and exhaled breath condensate of patients with lung cancer (Aleman et al., 2002; Fracchia et al., 1999; Shi et al., 2014). In patients with non-small-cell lung cancer (NSCLC), the increase in serum ferritin is attributed to inflammation and oxidative stress, rather than changes in body iron (Kukulj et al., 2010). Serum ferritin concentration is also closely associated with the prognosis and survival of patients with primary lung cancer (Milman N, 2002). Increased expression of TFR1 has been observed in 88% of tumours in patients with NSCLC (Kukulj et al., 2010), suggesting that lung cancer cells may increase iron uptake through increased TF:TFR1 interactions. A recent study has shown that epidermal growth factor receptor (EGFR) modulates cellular iron homeostasis through promoting increased cellular surface expression of TFR1 and that this is essential for cancer development and progression (Wang B, 2016). Interestingly, increased TF production has been reported in SCLC cells and is important for promoting cellular proliferation in an autocrine manner (Vostrejs et al., 1988). In other studies overexpression of LNC2 has been associated with radio-resistance of lung cancer cells and predicts worse survival of lung cancer patients (Ruiz-Morales et al., 2015; Shiiba et al., 2013). It has been reported that microRNAs (miRNAs) regulate the expression of several genes involved in iron homeostasis and that these miRNAs may play a role in the pathogenesis of lung cancer (Castoldi and Muckenthaler, 2012). The expression of FPN is downregulated by the binding

of miRNA (miR)-20a to highly conserved target sites in its 3' UTR. In individuals with pulmonary adenocarcinoma and squamous cell carcinoma a significant inverse correlation is observed between miR-20a levels and FPN expression (Babu and Muckenthaler, 2016). Furthermore, FPN is negatively regulated by miR-20a in NSCLC cells, with the manipulation of miR-20a or FPN capable of affecting colony formation and cellular proliferation. Ziolkowska-Suchanek *et al.*, have shown that IRP2 variants are associated with increased risk of lung cancer (Ziolkowska-Suchanek *et al.*, 2015). In addition, IRP2 has been shown to promote pro-oncogenic properties in human lung cancer cells (Maffettone *et al.*, 2010). Together, these findings suggest a role for dysregulated iron regulation and/or accumulation in cells in the pathogenesis of lung cancer and further highlight the therapeutic potential for targeting iron regulation in the treatment of lung disease. Additionally, humans and mice with COPD have altered microbiomes and increased susceptibility to respiratory infections that exacerbate their disease (Beckett *et al.*, 2013; Budden *et al.*, 2016; Chambers *et al.*, 2014; Hsu *et al.*, 2016; Hsu *et al.*, 2012; Hsu *et al.*, 2015; Jones *et al.*, 2016; King *et al.*, 2015; Simpson *et al.*, 2016; Simpson *et al.*, 2014; Singanayagam *et al.*, 2015; Starkey *et al.*, 2013b; Tay *et al.*, 2015), and increases in iron may be involved in these process.

5.3 Cystic fibrosis

CF is a fatal genetic lung disease caused by dysfunction of the anion transporter CF transmembrane conductance regulator (CFTR), resulting in reduced airway surface liquid and increased production of abnormally thick, viscous mucus in the CF lung (Boucher, 2007; O'Sullivan and Freedman, 2009). It also affects other organs including liver, pancreas, kidneys and intestine (O'Sullivan and Freedman, 2009). Along with a number of other symptoms, breathing difficulties, as a result of the accumulation of thick mucus in the airways, and frequent and chronic lung infections are major characteristic features of CF (O'Sullivan and Freedman, 2009). Abnormal iron homeostasis, including, systemic iron deficiency as well as dysregulation of intracellular molecular pathways associated with increased iron accumulation and defective iron sequestration in the airway cells have been found in experimental models of CF and human CF patients (Chillappagari *et al.*, 2014; Reid *et al.*, 2002; Uijterschout *et al.*, 2014). Altered iron homeostasis in the lower respiratory tract has also been associated with CF and severity of disease, with elevated levels of iron present in the (BAL), sputum and alveolar and interstitial macrophages from CF patients compared to healthy controls and increased iron levels correlating with declining lung function (Ghio *et al.*, 2013; Reid *et al.*, 2004; Reid *et al.*, 2002; Stites *et al.*, 1999). Furthermore, the increased

iron levels observed in the airways of CF patients may contribute to the increased susceptibility to the chronic bacterial infections that are associated with CF.

5.4 IPF

IPF is a chronic and fatal progressive lung disease characterised by persistent and progressive damage of alveoli and lung fibrosis, associated with an advanced decline in lung function that renders the lungs incapable of effectively facilitating oxygen transport into the bloodstream (Raghu et al., 2011). Whilst the exact causes of IPF are unclear, smoking and other environmental factors, such as exposure of dust, silica and infections, have been suggested to play roles in its development (Olson and Swigris, 2012; Williams, 2014). Recent studies have shown that total iron levels, the generation of iron-associated oxygen radicals and iron-laden macrophages are elevated in the lungs of IPF patients compared to controls (Puxeddu et al., 2014; Sangiuolo et al., 2015). Furthermore, accumulation of excess iron has been shown to be associated with vascular abnormalities, including pulmonary arterial hypertension, in the lungs of IPF patients (Colombat et al., 2007; Kim et al., 2010). These findings suggest that disruption of iron homeostasis and iron overloading may play a role in the pathogenesis of IPF.

5.5 Asthma

Asthma is a common obstructive lung disease that affects approximately 10% of the population in developed nations (Hansbro et al., 2008; Hansbro et al., 2004; Hansbro et al., 2011; Hansbro et al., 2014; Wang et al., 2010; Yang et al., 2012). It is underpinned by aberrant responses to otherwise harmless environmental stimuli that drive chronic inflammation, smooth muscle contraction and mucus hypersecretion in the airways that result in airways narrowing and the widespread but variable airflow obstruction that gives rise to the breathing difficulties associated with disease (Hansbro et al., 2008; Hansbro et al., 2004; Hansbro et al., 2011; Hansbro et al., 2014; Wang et al., 2010; Yang et al., 2012).

A relatively small number of clinical and experimental studies have reported evidence for altered systemic and lung levels of iron and iron regulatory molecules being associated with asthma. Clinically, lower iron levels have been reported to be associated with asthma in children and adults (Brigham et al., 2015; Ramakrishnan and Borade, 2010; Vlastic et al., 2009). However, it is not clear as to whether decreased iron levels play a role in the pathogenesis of asthma or are a consequence of disease given that chronic inflammatory responses are associated with decreased iron levels. Short-term intraperitoneal injections of

iron have been demonstrated to inhibit disease in an acute mouse model of asthma (Maazi et al., 2011), suggesting that increasing iron levels may be protect against disease, however, the potential pathological effects of longer-term injections of high concentrations of iron in the lung remain to be examined. Further studies investigating the effects of long term iron overloading with genetic and/or dietary models are required in order to appropriately address the long term consequences of iron loading on the asthmatic lung. Ferritin levels have been reported to be increased in the lung in an ovalbumin-induced mouse model of asthma (Bibi et al., 2014). Significantly, the levels of ferritin and ferritin-bound iron were shown to be decreased in this model by intranasal treatment with iron chelator complexes, with treatment resulting in an attenuation of asthma-associated inflammatory responses (Bibi et al., 2014). In another study, the administration of LF reduced inflammation in a ragweed-induced model of asthma (Kruzel et al., 2006), however, how the model affected iron levels in the lung and the effects that LF treatment had on these responses, were not explored in this study.

Whilst more studies investigating the link between iron levels and asthma are clearly required, these findings provide evidence that asthma is associated with altered iron homeostasis and suggest that correcting abnormal iron levels or iron regulatory molecule expression may be effective for the treatment of disease. It is also worth noting that the associations between iron levels and asthma may vary between different phenotypes of asthma and during exacerbations of disease (Hansbro et al., 2011). Considering that infections are associated with the development of asthma phenotype (Beckett et al., 2012; Hansbro et al., 2008; Hansbro et al., 2004; Horvat et al., 2007; Horvat et al., 2010b; Starkey et al., 2013a; Starkey et al., 2012; Starkey et al., 2016; Starkey et al., 2014) and that humans and mice with asthma or allergic airway disease have altered microbiomes (Arrieta et al., 2015; Marsland et al., 2015) and increased susceptibility to respiratory infections (Essilfie et al., 2015; Essilfie et al., 2012; Essilfie et al., 2011; Horvat et al., 2010a; Kim et al., 2016; Kim et al., 2015; Wood et al., 2010; Yang et al., 2012) that increase the severity or exacerbate their disease (Starkey et al., 2013b), alterations in iron homeostasis may be involved in these processes.

5.6 Other lung diseases

ARDS is a severe lung illness that leads to accumulation of fluid in the alveoli, which inhibits oxygen transfer to the blood. ARDS can result from trauma, inhalation of noxious particles, pneumonia and lung transplantation. It has been proposed that ARDS is associated with increased levels of iron, TF, TFR, LF and ferritin in BAL, increased serum ferritin BAL

levels and polymorphisms in iron regulatory genes (Ghio et al., 2003; Lagan et al., 2008; Sharkey et al., 1999).

Transfusion-related acute lung injury (TRALI) is a serious life-threatening complication and is the leading cause of blood transfusion-related morbidity and mortality (Peters et al., 2015). TRALI is induced by transfusion of blood products, which is known to elevate systemic iron levels in patients, resulting in oxidative stress and a number of pathological consequences (Collard, 2014; Dani et al., 2004; Gattermann and Rachmilewitz, 2011; Jenkins et al., 2007; Peters et al., 2015). Increased iron accumulation is also observed following lung transplantation, with higher levels of iron accumulation associated with increased risk of acute organ rejection (Baz et al., 1997; Reid et al., 2001; Sandmeier et al., 2005).

Pulmonary alveolar proteinosis (PAP) is characterised by abnormal accumulation of protein-rich surfactant and inflammation, with unusual accumulation in the lung of alveolar macrophages containing intracellular surfactant-like material and is associated with impaired lung function (Rosen et al., 1958; Shimizu et al., 2011). The BAL fluid of patients with primary or idiopathic PAP display elevated levels of iron, TF, TFR1, LF and ferritin and a reduction in the concentration of antioxidants such as glutathione, which may lead to an increase in iron-catalysed oxidative damage (Ghio et al., 2008b).

Whilst these studies suggest that altered iron accumulation and/or a disruption in iron homeostasis may be associated with a number of lung diseases, the nature of the interplay between dysregulated iron and the progression of disease remains to be elucidated in most cases. If iron levels in tissues saturate protective mechanisms, production of reactive oxygen species and induction of oxidative stress may arise. Therefore, increased accumulation of iron in the lung may contribute to a number of lung diseases by promoting oxidative stress, which can result in tissue damage, inflammation and altered lung structure and function and/or mitochondrial dysfunction (**Fig 3**). Furthermore, disruptions to iron availability may affect key biological processes important in maintaining normal cell function (**Fig 3**). It is also possible that altered iron levels may be a consequence of tissue damage, for example by releasing iron from storage reservoirs such as macrophages, or disruption of iron-regulatory mechanisms by disease processes (**Fig 3**). Further studies are required to investigate the mechanistic roles that dysregulated iron levels may play in the development of lung disease. The elucidation of such mechanisms may highlight novel therapeutic strategies that are based on correcting iron homeostasis in lung diseases, particularly those associated with evidence of dysregulated iron homeostasis.

6. Iron and the pathogenesis of respiratory infections

Iron and iron-regulatory proteins play an important role in the pathogenesis of a number of clinically significant respiratory infections, including, but not limited to, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*. Importantly, the relationships that exist between iron and respiratory infection may have important ramifications for the pathogenesis and treatment of a number of infection-associated lung diseases.

H. influenzae is a Gram negative respiratory tract pathogen that is associated with a number of clinically significant lung diseases, including asthma (particularly non-eosinophilic, steroid refractory forms of disease), COPD, CF and chronic bronchitis (Cardines et al., 2012; Essilfie et al., 2012; Kim et al., 2016; Simpson et al., 2016; Wood et al., 2010; Yi et al., 1997). *H. influenzae* expresses the siderophore, human TF-binding protein, which binds human TF and allows the bacterium to sequester iron required for growth (Schryvers and Gray-Owen, 1992; Vogel et al., 1997a). In addition to TF, *H. influenzae* can also source iron through the uptake of host proteins such as LF, ferritin and haemoglobin (Pidcock et al., 1988; Vogel et al., 1997a). Significantly, studies have shown that TF levels are increased in the sputum of patients with chronic bronchitis associated with *H. influenzae* infections (Vogel et al., 1997b). However, whether the increased TF levels are the consequence of chronic bronchitis or are the cause of chronic infection and, therefore, the driver of disease, remains to be elucidated. Vaccination with recombinant TF binding proteins, which induce antibody responses that block *H. influenzae* surface expressed TF binding proteins and ability to sequester TF, has been shown to be effective for protecting against non-typeable *H. influenzae* (Webb and Cripps, 1999). This suggests that therapies that target TF binding or other bacterial means for sourcing iron may be effective for the treatment of *H. influenzae* infections associated with chronic respiratory disease.

The Gram-positive respiratory pathogen, *S. pneumoniae* is responsible for a number of respiratory tract diseases including pneumonia and otitis media, as well as septicaemia and meningitis (Mitchell, 2000). Iron has been shown to be an important factor required for *S. pneumoniae* growth and replication, both *in vitro* and *in vivo* (Brown et al., 2001a; Brown et al., 2002; Brown et al., 2001b). To sequester iron, *S. pneumoniae* can utilize several iron sources such as hemoglobin, hemin, ferritin, ferric and ferrous iron salts, ferrioxamine and xenosiderophore (Brown et al., 2001a; Brown et al., 2001b; Ge and Sun, 2014; Gupta et al., 2009; Romero-Espejel et al., 2013; Tai et al., 1993). In addition to *S. pneumoniae*, iron has been shown to be critically important in the pathogenesis of pneumonia caused by *S. aureus*

(Mason and Skaar, 2009) and *Klebsiella pneumoniae* (Bachman et al., 2012). *S. aureus* acquires haem-iron *via* the haem transport and the iron-regulated surface determinant systems (Mazmanian et al., 2003; Muryoi et al., 2008; Skaar et al., 2004a; Skaar et al., 2004b). *K. pneumoniae* needs to secrete siderophores for bacterial replication and virulence (Bachman et al., 2015; Holden et al., 2016; Lawlor et al., 2007). It has been reported that *K. pneumoniae* siderophores chelate epithelial cell iron, promote cytokine secretion and activate the master transcription factor, hypoxia inducible factor-1 α (HIF-1 α), which regulates vascular permeability and inflammatory gene expression (Holden et al., 2014; Nelson et al., 2007). Indeed, siderophores secreted by *K. pneumoniae* have been suggested to act directly on the host to induce inflammatory cytokines and mediate bacterial dissemination, with HIF-1 α a susceptibility factor for bacterial invasion during pneumonia (Holden et al., 2016; Holden et al., 2014). Furthermore, increased levels of iron-storing macrophages have been shown in the BAL fluid of patients with severe bacterial pneumonia (Grigoriu et al., 2006). Together, these findings suggest that targeting iron availability in the lung may be effective for the management of pneumonia caused by these bacteria.

M. tuberculosis is an intracellular bacterial pathogen that infects macrophages within the lung and is the major causative agent of tuberculosis. *M. tuberculosis* secretes siderophores that diffuse out of the phagosome/inclusion of infected macrophages in order to source cytoplasmic iron (Luo et al., 2005). *M. tuberculosis* can also decrease FPN cell surface expression in lung macrophages, which may enhance intracellular iron levels in order to facilitate replication (Van Zandt et al., 2008). Additionally, *M. tuberculosis* can source iron from exogenous TF, as it cycles through the TF/TFR1 endocytic pathway, through interactions between the *M. tuberculosis* phagosome and early endosomes (Clemens and Horwitz, 1996; Olakanmi et al., 2002). An elevated level of dietary iron has been shown to increase susceptibility to *M. tuberculosis* infections (Gangaidzo et al., 2001). Importantly, targeting iron availability and sequestration has been shown to be effective for reducing *Mycobacteria* infections (Schaible et al., 2002). Inhibition of TFR1 and TF uptake has been shown to inhibit the growth of *M. tuberculosis* as well as another intracellular pathogen, *Legionella pneumophila* (Byrd and Horwitz, 1989; Olakanmi et al., 2002). The growth and replication of *M. tuberculosis* within macrophages can also be inhibited by deleting the bacterial genes that code for siderophore production (De Voss et al., 2000), providing evidence that siderophore biosynthesis could be a potential target for new antimicrobial agents for pulmonary tuberculosis. Certainly, a mycobacterial-specific siderophore analog

conjugated with the antimalarial agent, artemisinin, has been shown to significantly inhibit *M. tuberculosis* replication *in vitro* (Miller et al., 2011).

P. aeruginosa, *H. influenzae* and *S. aureus* are the most commonly identified pathogens found in the lungs of CF patients and are major complicating factors that contribute to the pathogenesis of CF (Davies and Bilton, 2009; Konings et al., 2013; Lamont et al., 2009). *P. aeruginosa* has multiple active iron uptake systems, including haem transport systems (Minandri et al., 2016). Indeed, many *P. aeruginosa* genes are iron-responsive, and code for factors that help to produce siderophores that sequester iron from the host (Minandri et al., 2016; Vasil and Ochsner, 1999). Increased bioavailability of iron stimulates *P. aeruginosa* biofilm formation, aggregation, adhesion and invasion, allowing the pathogen to avoid host removal (Berlutti et al., 2005). Whilst iron deficiency is frequently observed in both adults and children with CF (Pond et al., 1996; Uijterschout et al., 2014), there is a significant increase in iron and ferritin in the sputum of CF patients, regardless of whether they are infected or not infected with *P. aeruginosa* (Stites et al., 1998). Indeed, free iron concentration in the mucosa and lower respiratory tract of healthy individuals is very low (10^{-18} M), whereas iron concentration in CF patient sputum is very high (6.3×10^{-5} M) (Berlutti et al., 2005). Significantly, in clinically stable CF patients, there is a strong positive correlation between sputum iron and *P. aeruginosa* levels (Reid et al., 2007). Together these findings have led to the suggestion that enhanced airway iron in CF patients is a potential causal factor of *P. aeruginosa* persistence in CF (Reid et al., 2007). These findings highlight the potential for correcting iron levels in the airways of CF patients as a potential therapeutic strategy for treating *P. aeruginosa* as well as *H. influenzae* and other infections that are commonly associated with the disease.

The human body has developed a number of strategies to restrict iron availability to pathogens and the lung is no exception. It has been demonstrated that LF blocks *P. aeruginosa* biofilm formation, provides protection against infection, and can reduce increased *M. tuberculosis* infectivity under iron overloading conditions (Schaible et al., 2002; Singh et al., 2002; Smith et al., 2013b). LF has also been shown to decrease the pathogenicity of a number of bacterial and viral lung infections (Grover et al., 1997; Pietrantonio et al., 2010; Welsh et al., 2011) and increase the efficacy of antimicrobial therapy for *P. aeruginosa*, with evidence suggesting that the effects of LF may be due to immunomodulation and/or the depletion of iron required for microbial replication (Frioni et al., 2014; Hwang et al., 2009; Marshall et al., 2016; Shin et al., 2005; Wakabayashi et al., 2014; Welsh et al., 2011). Interestingly, Britigan *et al.*, have shown that LF is cleaved in the lungs of *P. aeruginosa*-

infected CF patients (Britigan et al., 1993), which may provide another potential mechanism for increased susceptibility to infection in CF.

Iron regulatory molecule expression is also modified in the lung by infection-induced immune responses, helping to combat infection. Up-regulation of DMT1 in bronchial epithelial cells is stimulated by a number of pro-inflammatory cytokines, including TNF- α and IFN- γ , which are produced during infection (Wang et al., 2005). This suggests immune responses may increase DMT1 expression in the lung during an infection, facilitating the uptake of iron (Wang et al., 2005). As explained in Section 3, there is a strong link between hepcidin and immunity. It has been suggested that immune-mediated regulation of hepcidin occurs in the lung, with IFN- γ inducing the expression of hepcidin in airway epithelial cells but apparently without changing iron levels (Frazier et al., 2011).

Increased expression of *slc11a1*/NRAMP1 decreases iron levels in phagosomes and inhibits intraphagosomal microbial infection in macrophages (Forbes and Gros, 2001; Jabado et al., 2000). Allelic variants at the human *Slc11a1* locus have been reported to be associated with susceptibility to *M. tuberculosis* and pulmonary *M. avium complex* infection and infection-induced disease (Archer et al., 2015; Gallant et al., 2007; Govoni and Gros, 1998; Sapkota et al., 2012; Tanaka et al., 2007; Zhang et al., 2005).

As explained in Section 3, LCN2 inhibits the growth of pathogens by interfering with siderophore-mediated iron sequestration (Chan et al., 2009; Cowland et al., 2003; Flo et al., 2004; Goetz et al., 2002; Holmes et al., 2005). LCN2 secreted by neutrophils and epithelial cells also appears to play an important role in the lung during infection (Bachman et al., 2012; Guglani et al., 2012; Martineau et al., 2007; Wu et al., 2010). LCN2 binds to siderophores produced by *K. pneumoniae*, blocking iron uptake and mediating protection against invasive lung infection (Bachman et al., 2012). It also inhibits *M. tuberculosis* replication in an iron-dependent manner (Martineau et al., 2007). Furthermore, serum LCN2 levels have been shown to be inversely correlated with *Mycobacteria* infection in patients with active pulmonary tuberculosis (Martineau et al., 2007) and *E. coli*-induced pneumonia has been shown to be exacerbated in LCN2 deficient mice (Wu et al., 2010). Together these findings suggest that LCN2 limits iron acquisition by pathogens and may protect the lung from microbial infection.

Conclusions

It is clear that a number of acute and chronic lung diseases are associated with altered iron levels and/or iron homeostasis in the lung and that iron plays an important role in

pathogenesis of several clinically significant respiratory infections. Interestingly, since a number of chronic lung conditions such as COPD, IPF, CF and severe forms of asthma are associated with respiratory infections, dysregulated iron homeostasis and/or deficiencies in protective iron regulatory mechanisms may provide a critical link to explain the association between infection and the pathogenesis of a number of infection-associated lung diseases (**Fig 3**). Whatever the relationships, the studies outlined in this review highlight the need for further investigation into the precise nature of the interplay between iron, pathology, infection and immunity in the context of respiratory disease in order to inform the mechanisms involved and explore the potential for therapeutic strategies that target iron, iron-regulatory molecules or iron-associated effects.

Conflict of interest

Authors declare no conflict of interest.

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References

- Actor, J.K., Hwang, S.A., Kruzel, M.L., 2009. Lactoferrin as a natural immune modulator. *Curr Pharm Des* 15, 1956-1973.
- Alberg, A.J., Ford, J.G., Samet, J.M., American College of Chest, P., 2007. Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). *Chest* 132, 29S-55S.
- Aleman, M.R., Santolaria, F., Batista, N., de La Vega, M., Gonzalez-Reimers, E., Milena, A., Llanos, M., Gomez-Sirvent, J.L., 2002. Leptin role in advanced lung cancer. A mediator of the acute phase response or a marker of the status of nutrition? *Cytokine* 19, 21-26.
- Anderson, G.J., 2007. Mechanisms of iron loading and toxicity. *Am J Hematol* 82, 1128-1131.
- Andrews, N.C., Schmidt, P.J., 2007. Iron homeostasis. *Annu Rev Physiol* 69, 69-85.
- Archer, N.S., Nassif, N.T., O'Brien, B.A., 2015. Genetic variants of SLC11A1 are associated with both autoimmune and infectious diseases: systematic review and meta-analysis. *Genes Immun* 16, 275-283.
- Armitage, A.E., Eddowes, L.A., Gileadi, U., Cole, S., Spottiswoode, N., Selvakumar, T.A., Ho, L.P., Townsend, A.R., Drakesmith, H., 2011. Hepcidin regulation by innate immune and infectious stimuli. *Blood* 118, 4129-4139.
- Arrieta, M.C., Stiemsma, L.T., Dimitriu, P.A., Thorson, L., Russell, S., Yurist-Doutsch, S., Kuzeljevic, B., Gold, M.J., Britton, H.M., Lefebvre, D.L., Subbarao, P., Mandhane, P., Becker, A., McNagny, K.M.,

Sears, M.R., et al., 2015. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med* 7, 307ra152.

Babu, K.R., Muckenthaler, M.U., 2016. miR-20a regulates expression of the iron exporter ferroportin in lung cancer. *J Mol Med (Berl)* 94, 347-359.

Bachman, M.A., Breen, P., Deornellas, V., Mu, Q., Zhao, L., Wu, W., Cavalcoli, J.D., Mobley, H.L., 2015. Genome-Wide Identification of *Klebsiella pneumoniae* Fitness Genes during Lung Infection. *MBio* 6, e00775.

Bachman, M.A., Lenio, S., Schmidt, L., Oyler, J.E., Weiser, J.N., 2012. Interaction of lipocalin 2, transferrin, and siderophores determines the replicative niche of *Klebsiella pneumoniae* during pneumonia. *MBio* 3.

Basaraba, R.J., Bielefeldt-Ohmann, H., Eschelbach, E.K., Reisenhauer, C., Tolnay, A.E., Taraba, L.C., Shanley, C.A., Smith, E.A., Bedwell, C.L., Chlipala, E.A., Orme, I.M., 2008. Increased expression of host iron-binding proteins precedes iron accumulation and calcification of primary lung lesions in experimental tuberculosis in the guinea pig. *Tuberculosis (Edinb)* 88, 69-79.

Baveye, S., Ellass, E., Mazurier, J., Spik, G., Legrand, D., 1999. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. *Clin Chem Lab Med* 37, 281-286.

Baz, M.A., Ghio, A.J., Roggli, V.L., Tapson, V.F., Piantadosi, C.A., 1997. Iron accumulation in lung allografts after transplantation. *Chest* 112, 435-439.

Beard, J.L., 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* 131, 568S-579S; discussion 580S.

Beckett, E.L., Phipps, S., Starkey, M.R., Horvat, J.C., Beagley, K.W., Foster, P.S., Hansbro, P.M., 2012. TLR2, but not TLR4, is required for effective host defence against *Chlamydia* respiratory tract infection in early life. *PLoS One* 7, e39460.

Beckett, E.L., Stevens, R.L., Jarnicki, A.G., Kim, R.Y., Hanish, I., Hansbro, N.G., Deane, A., Keely, S., Horvat, J.C., Yang, M., Oliver, B.G., van Rooijen, N., Inman, M.D., Adachi, R., Soberman, R.J., Hamadi, S., Wark, P.A., Foster, P.S., Hansbro, P.M., 2013. A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis. *J Allergy Clin Immunol* 131, 752-762.

Berlutti, F., Morea, C., Battistoni, A., Sarli, S., Cipriani, P., Superti, F., Ammendolia, M.G., Valenti, P., 2005. Iron availability influences aggregation, biofilm, adhesion and invasion of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*. *Int J Immunopathol Pharmacol* 18, 661-670.

Bibi, H., Vinokur, V., Waisman, D., Elenberg, Y., Landesberg, A., Faingersh, A., Yadid, M., Brod, V., Pesin, J., Berenshtein, E., Eliashar, R., Chevion, M., 2014. Zn/Ga-DFO iron-chelating complex attenuates the inflammatory process in a mouse model of asthma. *Redox Biol* 2, 814-819.

Boucher, R.C., 2007. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. *Annu Rev Med* 58, 157-170.

Bridle, K.R., Frazer, D.M., Wilkins, S.J., Dixon, J.L., Purdie, D.M., Crawford, D.H., Subramaniam, V.N., Powell, L.W., Anderson, G.J., Ramm, G.A., 2003. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet* 361, 669-673.

Brigham, E.P., McCormack, M.C., Takemoto, C.M., Matsui, E.C., 2015. Iron status is associated with asthma and lung function in US women. *PLoS One* 10, e0117545.

Britigan, B.E., Hayek, M.B., Doebbeling, B.N., Fick, R.B., Jr., 1993. Transferrin and lactoferrin undergo proteolytic cleavage in the *Pseudomonas aeruginosa*-infected lungs of patients with cystic fibrosis. *Infect Immun* 61, 5049-5055.

Brown, J.S., Gilliland, S.M., Holden, D.W., 2001a. A *Streptococcus pneumoniae* pathogenicity island encoding an ABC transporter involved in iron uptake and virulence. *Mol Microbiol* 40, 572-585.

Brown, J.S., Gilliland, S.M., Ruiz-Albert, J., Holden, D.W., 2002. Characterization of pit, a *Streptococcus pneumoniae* iron uptake ABC transporter. *Infect Immun* 70, 4389-4398.

Brown, J.S., Ogunniyi, A.D., Woodrow, M.C., Holden, D.W., Paton, J.C., 2001b. Immunization with components of two iron uptake ABC transporters protects mice against systemic *Streptococcus pneumoniae* infection. *Infect Immun* 69, 6702-6706.

Budden, K.F., Gellatly, S.L., Wood, D.L., Cooper, M.A., Morrison, M., Hugenholtz, P., Hansbro, P.M., 2016. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol*.

Bullen, J.J., Spalding, P.B., Ward, C.G., Gutteridge, J.M., 1991. Hemochromatosis, iron and septicemia caused by *Vibrio vulnificus*. *Arch Intern Med* 151, 1606-1609.

Byrd, T.F., Horwitz, M.A., 1989. Interferon gamma-activated human monocytes downregulate transferrin receptors and inhibit the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. *J Clin Invest* 83, 1457-1465.

Byrd, T.F., Horwitz, M.A., 1993. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. *J Clin Invest* 91, 969-976.

Cappellini MD, C.A., Eleftheriou A, Piga A, Porter J, Taher A, 2008. Guidelines for the Clinical Management of Thalassaemia [Internet]. 2nd Revised edition. Nicosia (CY): Thalassaemia International Federation.

Cardines, R., Giufre, M., Pompilio, A., Fiscarelli, E., Ricciotti, G., Di Bonaventura, G., Cerquetti, M., 2012. *Haemophilus influenzae* in children with cystic fibrosis: antimicrobial susceptibility, molecular epidemiology, distribution of adhesins and biofilm formation. *Int J Med Microbiol* 302, 45-52.

Caron, J., Loredano-Osti, J.C., Laroche, L., Skamene, E., Morgan, K., Malo, D., 2002. Identification of genetic loci controlling bacterial clearance in experimental *Salmonella enteritidis* infection: an unexpected role of Nramp1 (Slc11a1) in the persistence of infection in mice. *Genes Immun* 3, 196-204.

Casanovas, G., Mleczko-Sanecka, K., Altamura, S., Hentze, M.W., Muckenthaler, M.U., 2009. Bone morphogenetic protein (BMP)-responsive elements located in the proximal and distal hepcidin promoter are critical for its response to HJV/BMP/SMAD. *J Mol Med (Berl)* 87, 471-480.

Cassat, J.E., Skaar, E.P., 2013. Iron in infection and immunity. *Cell Host Microbe* 13, 509-519.

Castoldi, M., Muckenthaler, M.U., 2012. Regulation of iron homeostasis by microRNAs. *Cell Mol Life Sci* 69, 3945-3952.

Chambers, D.C., Gellatly, S.L., Hugenholtz, P., Hansbro, P.M., 2014. JTD special edition 'Hot Topics in COPD'-The microbiome in COPD. *J Thorac Dis* 6, 1525-1531.

Chan, Y.R., Liu, J.S., Pociask, D.A., Zheng, M., Mietzner, T.A., Berger, T., Mak, T.W., Clifton, M.C., Strong, R.K., Ray, P., Kolls, J.K., 2009. Lipocalin 2 is required for pulmonary host defense against *Klebsiella* infection. *J Immunol* 182, 4947-4956.

Chappell, S.L., Daly, L., Lotya, J., Alsaegh, A., Guetta-Baranes, T., Roca, J., Rabinovich, R., Morgan, K., Millar, A.B., Donnelly, S.C., Keatings, V., MacNee, W., Stolk, J., Hiemstra, P.S., Miniati, M., Monti, S., O'Connor, C.M., Kalsheker, N., 2011. The role of IREB2 and transforming growth factor beta-1 genetic variants in COPD: a replication case-control study. *BMC Med Genet* 12, 24.

Chillappagari, S., Venkatesan, S., Garapati, V., Mahavadi, P., Munder, A., Seubert, A., Sarode, G., Guenther, A., Schmeck, B.T., Tummler, B., Henke, M.O., 2014. Impaired TLR4 and HIF expression in cystic fibrosis bronchial epithelial cells downregulates hemoxygenase-1 and alters iron homeostasis in vitro. *Am J Physiol Lung Cell Mol Physiol* 307, L791-799.

Christopher, G.W., 1985. *Escherichia coli* bacteremia, meningitis, and hemochromatosis. *Arch Intern Med* 145, 1908.

Clemens, D.L., Horwitz, M.A., 1996. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin. *J Exp Med* 184, 1349-1355.

Cloonan, S.M., Glass, K., Laucho-Contreras, M.E., Bhashyam, A.R., Cervo, M., Pabon, M.A., Konrad, C., Polverino, F., Siempos, I, Perez, E., Mizumura, K., Ghosh, M.C., Parameswaran, H., Williams, N.C., Rooney, K.T., et al., 2016. Mitochondrial iron chelation ameliorates cigarette smoke-induced bronchitis and emphysema in mice. *Nat Med* 22, 163-174.

Collard, K.J., 2014. Transfusion related morbidity in premature babies: Possible mechanisms and implications for practice. *World J Clin Pediatr* 3, 19-29.

Colombat, M., Mal, H., Groussard, O., Capron, F., Thabut, G., Jebrak, G., Brugiere, O., Dauriat, G., Castier, Y., Leseche, G., Fournier, M., 2007. Pulmonary vascular lesions in end-stage idiopathic pulmonary fibrosis: Histopathologic study on lung explant specimens and correlations with pulmonary hemodynamics. *Hum Pathol* 38, 60-65.

Core, A.B., Canali, S., Babitt, J.L., 2014. Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis. *Front Pharmacol* 5, 104.

Corhay, J.L., Weber, G., Bury, T., Mariz, S., Roelandts, I., Radermecker, M.F., 1992. Iron content in human alveolar macrophages. *Eur Respir J* 5, 804-809.

Cowland, J.B., Sorensen, O.E., Sehested, M., Borregaard, N., 2003. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. *J Immunol* 171, 6630-6639.

Dani, C., Martelli, E., Bertini, G., Pezzati, M., Rossetti, M., Buonocore, G., Paffetti, P., Rubaltelli, F.F., 2004. Effect of blood transfusions on oxidative stress in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 89, F408-411.

Davies, J.C., Bilton, D., 2009. Bugs, biofilms, and resistance in cystic fibrosis. *Respir Care* 54, 628-640.

De Voss, J.J., Rutter, K., Schroeder, B.G., Su, H., Zhu, Y., Barry, C.E., 3rd, 2000. The salicylate-derived mycobactin siderophores of *Mycobacterium tuberculosis* are essential for growth in macrophages. *Proc Natl Acad Sci U S A* 97, 1252-1257.

DeMeo, D.L., Mariani, T., Bhattacharya, S., Srisuma, S., Lange, C., Litonjua, A., Bueno, R., Pillai, S.G., Lomas, D.A., Sparrow, D., Shapiro, S.D., Criner, G.J., Kim, H.P., Chen, Z., Choi, A.M., Reilly, J., Silverman, E.K., 2009. Integration of genomic and genetic approaches implicates IREB2 as a COPD susceptibility gene. *Am J Hum Genet* 85, 493-502.

Drago-Serrano, M.E., Rivera-Aguilar, V., Resendiz-Albor, A.A., Campos-Rodriguez, R., 2010. Lactoferrin increases both resistance to *Salmonella typhimurium* infection and the production of antibodies in mice. *Immunol Lett* 134, 35-46.

Eagan, T.M., Damas, J.K., Ueland, T., Voll-Aanerud, M., Mollnes, T.E., Hardie, J.A., Bakke, P.S., Aukrust, P., 2010. Neutrophil gelatinase-associated lipocalin: a biomarker in COPD. *Chest* 138, 888-895.

Engstrom, G., Segelstorm, N., Ekberg-Aronsson, M., Nilsson, P.M., Lindgarde, F., Lofdahl, C.G., 2009. Plasma markers of inflammation and incidence of hospitalisations for COPD: results from a population-based cohort study. *Thorax* 64, 211-215.

Erlitzki, R., Long, J.C., Theil, E.C., 2002. Multiple, conserved iron-responsive elements in the 3'-untranslated region of transferrin receptor mRNA enhance binding of iron regulatory protein 2. *J Biol Chem* 277, 42579-42587.

Essilfie, A.T., Horvat, J.C., Kim, R.Y., Mayall, J.R., Pinkerton, J.W., Beckett, E.L., Starkey, M.R., Simpson, J.L., Foster, P.S., Gibson, P.G., Hansbro, P.M., 2015. Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma. *Thorax* 70, 458-467.

Essilfie, A.T., Simpson, J.L., Dunkley, M.L., Morgan, L.C., Oliver, B.G., Gibson, P.G., Foster, P.S., Hansbro, P.M., 2012. Combined *Haemophilus influenzae* respiratory infection and allergic airways disease drives chronic infection and features of neutrophilic asthma. *Thorax* 67, 588-599.

Essilfie, A.T., Simpson, J.L., Horvat, J.C., Preston, J.A., Dunkley, M.L., Foster, P.S., Gibson, P.G., Hansbro, P.M., 2011. *Haemophilus influenzae* infection drives IL-17-mediated neutrophilic allergic airways disease. *PLoS Pathog* 7, e1002244.

Fernandez, J.M., Serrano, M., De Arriba, J.J., Sanchez, M.V., Escibano, E., Ferreras, P., 2000. Bacteremic cellulitis caused by Non-01, Non-0139 *Vibrio cholerae*: report of a case in a patient with hemochromatosis. *Diagn Microbiol Infect Dis* 37, 77-80.

Fischer, R., Debbabi, H., Dubarry, M., Boyaka, P., Tome, D., 2006. Regulation of physiological and pathological Th1 and Th2 responses by lactoferrin. *Biochem Cell Biol* 84, 303-311.

Fleming, R.E., Ahmann, J.R., Migas, M.C., Waheed, A., Koeffler, H.P., Kawabata, H., Britton, R.S., Bacon, B.R., Sly, W.S., 2002. Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. *Proc Natl Acad Sci U S A* 99, 10653-10658.

Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., Akira, S., Aderem, A., 2004. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 432, 917-921.

Forbes, J.R., Gros, P., 2001. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. *Trends Microbiol* 9, 397-403.

Foster, P.S., Plank, M., Collison, A., Tay, H.L., Kaiko, G.E., Li, J., Johnston, S.L., Hansbro, P.M., Kumar, R.K., Yang, M., Mattes, J., 2013. The emerging role of microRNAs in regulating immune and inflammatory responses in the lung. *Immunol Rev* 253, 198-215.

Fracchia, A., Ubbiali, A., El Bitar, O., Pacetti, M., Sommariva, E., Arreghini, M., Longhini, E., Bonalumi, G.P., 1999. A comparative study on ferritin concentration in serum and bilateral bronchoalveolar lavage fluid of patients with peripheral lung cancer versus control subjects. *Oncology* 56, 181-188.

Frazier, M.D., Mamo, L.B., Ghio, A.J., Turi, J.L., 2011. Hfe expression in human airway epithelial cells is regulated by interferon-gamma. *Respir Res* 12, 100.

Fricker, M., Deane, A., Hansbro, P.M., 2014. Animal models of chronic obstructive pulmonary disease. *Expert Opin Drug Discov* 9, 629-645.

Froni, A., Conte, M.P., Cutone, A., Longhi, C., Musci, G., di Patti, M.C., Natalizi, T., Marazzato, M., Lepanto, M.S., Puddu, P., Paesano, R., Valenti, P., Berlutti, F., 2014. Lactoferrin differently modulates the inflammatory response in epithelial models mimicking human inflammatory and infectious diseases. *Biometals* 27, 843-856.

Fritsche, G., Nairz, M., Theurl, I., Mair, S., Bellmann-Weiler, R., Barton, H.C., Weiss, G., 2007. Modulation of macrophage iron transport by Nramp1 (Slc11a1). *Immunobiology* 212, 751-757.

Fritsche, G., Nairz, M., Werner, E.R., Barton, H.C., Weiss, G., 2008. Nramp1-functionality increases iNOS expression via repression of IL-10 formation. *Eur J Immunol* 38, 3060-3067.

Gallant, C.J., Malik, S., Jabado, N., Cellier, M., Simkin, L., Finlay, B.B., Graviss, E.A., Gros, P., Musser, J.M., Schurr, E., 2007. Reduced in vitro functional activity of human NRAMP1 (SLC11A1) allele that predisposes to increased risk of pediatric tuberculosis disease. *Genes Immun* 8, 691-698.

Gammella, E., Buratti, P., Cairo, G., Recalcati, S., 2014. Macrophages: central regulators of iron balance. *Metallomics* 6, 1336-1345.

Gangaidzo, I.T., Moyo, V.M., Mvundura, E., Aggrey, G., Murphree, N.L., Khumalo, H., Saungweme, T., Kasvosve, I., Gomo, Z.A., Rouault, T., Boelaert, J.R., Gordeuk, V.R., 2001. Association of pulmonary tuberculosis with increased dietary iron. *J Infect Dis* 184, 936-939.

Ganz, T., 2012. Macrophages and systemic iron homeostasis. *J Innate Immun* 4, 446-453.

Ganz, T., Nemeth, E., 2006. Iron imports. IV. Hfe and regulation of body iron metabolism. *Am J Physiol Gastrointest Liver Physiol* 290, G199-203.

Gao, J., Chen, J., De Domenico, I., Koeller, D.M., Harding, C.O., Fleming, R.E., Koeberl, D.D., Enns, C.A., 2010. Hepatocyte-targeted HFE and TFR2 control hepcidin expression in mice. *Blood* 115, 3374-3381.

Gattermann, N., Rachmilewitz, E.A., 2011. Iron overload in MDS-pathophysiology, diagnosis, and complications. *Ann Hematol* 90, 1-10.

Ge, R., Sun, X., 2014. Iron acquisition and regulation systems in *Streptococcus* species. *Metallomics* 6, 996-1003.

Gehrke, S.G., Kulaksiz, H., Herrmann, T., Riedel, H.D., Bents, K., Veltkamp, C., Stremmel, W., 2003. Expression of hepcidin in hereditary hemochromatosis: evidence for a regulation in response to the serum transferrin saturation and to non-transferrin-bound iron. *Blood* 102, 371-376.

Geissler, C., Singh, M., 2011. Iron, meat and health. *Nutrients* 3, 283-316.

Ghio, A.J., Carter, J.D., Dailey, L.A., Devlin, R.B., Samet, J.M., 1999. Respiratory epithelial cells demonstrate lactoferrin receptors that increase after metal exposure. *Am J Physiol* 276, L933-940.

Ghio, A.J., Carter, J.D., Richards, J.H., Richer, L.D., Grissom, C.K., Elstad, M.R., 2003. Iron and iron-related proteins in the lower respiratory tract of patients with acute respiratory distress syndrome. *Crit Care Med* 31, 395-400.

Ghio, A.J., Carter, J.D., Samet, J.M., Reed, W., Quay, J., Dailey, L.A., Richards, J.H., Devlin, R.B., 1998. Metal-dependent expression of ferritin and lactoferrin by respiratory epithelial cells. *Am J Physiol* 274, L728-736.

Ghio, A.J., Hilborn, E.D., Stonehuerner, J.G., Dailey, L.A., Carter, J.D., Richards, J.H., Crissman, K.M., Foronjy, R.F., Uyeminami, D.L., Pinkerton, K.E., 2008a. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med* 178, 1130-1138.

Ghio, A.J., Roggli, V.L., Soukup, J.M., Richards, J.H., Randell, S.H., Muhlebach, M.S., 2013. Iron accumulates in the lavage and explanted lungs of cystic fibrosis patients. *J Cyst Fibros* 12, 390-398.

Ghio, A.J., Stonehuerner, J.G., Richards, J.H., Crissman, K.M., Roggli, V.L., Piantadosi, C.A., Carraway, M.S., 2008b. Iron homeostasis and oxidative stress in idiopathic pulmonary alveolar proteinosis: a case-control study. *Respir Res* 9, 10.

Ghio, A.J., Turi, J.L., Yang, F., Garrick, L.M., Garrick, M.D., 2006. Iron homeostasis in the lung. *Biol Res* 39, 67-77.

Giorgi, G., D'Anna, M.C., Roque, M.E., 2015. Iron homeostasis and its disruption in mouse lung in iron deficiency and overload. *Exp Physiol* 100, 1199-1216.

Goetz, D.H., Holmes, M.A., Borregaard, N., Bluhm, M.E., Raymond, K.N., Strong, R.K., 2002. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 10, 1033-1043.

Govoni, G., Gros, P., 1998. Macrophage NRAMP1 and its role in resistance to microbial infections. *Inflamm Res* 47, 277-284.

Gozzelino, R., Arosio, P., 2016. Iron Homeostasis in Health and Disease. *Int J Mol Sci* 17.

Green, N.S., 1992. Yersinia infections in patients with homozygous beta-thalassemia associated with iron overload and its treatment. *Pediatr Hematol Oncol* 9, 247-254.

Grigoriu, B., Jacobs, F., Beuzen, F., El Khoury, R., Axler, O., Brivet, F.G., Capron, F., 2006. Bronchoalveolar lavage cytological alveolar damage in patients with severe pneumonia. *Crit Care* 10, R2.

Grover, M., Giouzeppos, O., Schnagl, R.D., May, J.T., 1997. Effect of human milk prostaglandins and lactoferrin on respiratory syncytial virus and rotavirus. *Acta Paediatr* 86, 315-316.

Gruenheid, S., Pinner, E., Desjardins, M., Gros, P., 1997. Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. *J Exp Med* 185, 717-730.

Guglani, L., Gopal, R., Rangel-Moreno, J., Junecko, B.F., Lin, Y., Berger, T., Mak, T.W., Alcorn, J.F., Randall, T.D., Reinhart, T.A., Chan, Y.R., Khader, S.A., 2012. Lipocalin 2 regulates inflammation during pulmonary mycobacterial infections. *PLoS One* 7, e50052.

Gunshin, H., Mackenzie, B., Berger, U.V., Gunshin, Y., Romero, M.F., Boron, W.F., Nussberger, S., Gollan, J.L., Hediger, M.A., 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388, 482-488.

Gupta, R., Shah, P., Swiatlo, E., 2009. Differential gene expression in *Streptococcus pneumoniae* in response to various iron sources. *Microb Pathog* 47, 101-109.

Hallstrand, T.S., Hackett, T.L., Altemeier, W.A., Matute-Bello, G., Hansbro, P.M., Knight, D.A., 2014. Airway epithelial regulation of pulmonary immune homeostasis and inflammation. *Clin Immunol* 151, 1-15.

Hansbro, N.G., Horvat, J.C., Wark, P.A., Hansbro, P.M., 2008. Understanding the mechanisms of viral induced asthma: new therapeutic directions. *Pharmacol Ther* 117, 313-353.

Hansbro, P.M., Beagley, K.W., Horvat, J.C., Gibson, P.G., 2004. Role of atypical bacterial infection of the lung in predisposition/protection of asthma. *Pharmacol Ther* 101, 193-210.

Hansbro, P.M., Kaiko, G.E., Foster, P.S., 2011. Cytokine/anti-cytokine therapy - novel treatments for asthma? *Br J Pharmacol* 163, 81-95.

Hansbro, P.M., Scott, G.V., Essilfie, A.T., Kim, R.Y., Starkey, M.R., Nguyen, D.H., Allen, P.D., Kaiko, G.E., Yang, M., Horvat, J.C., Foster, P.S., 2013. Th2 cytokine antagonists: potential treatments for severe asthma. *Expert Opin Investig Drugs* 22, 49-69.

Hansbro, P.M., Starkey, M.R., Mattes, J., Horvat, J.C., 2014. Pulmonary immunity during respiratory infections in early life and the development of severe asthma. *Ann Am Thorac Soc* 11 Suppl 5, S297-302.

Hanson, C., Rutten, E.P., Wouters, E.F., Rennard, S., 2014. Influence of diet and obesity on COPD development and outcomes. *Int J Chron Obstruct Pulmon Dis* 9, 723-733.

Hentze, M.W., Muckenthaler, M.U., Andrews, N.C., 2004. Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117, 285-297.

Hoen, B., Kessler, M., Hestin, D., Mayeux, D., 1995. Risk factors for bacterial infections in chronic haemodialysis adult patients: a multicentre prospective survey. *Nephrol Dial Transplant* 10, 377-381.

Holden, V.I., Breen, P., Houle, S., Dozois, C.M., Bachman, M.A., 2016. Klebsiella pneumoniae Siderophores Induce Inflammation, Bacterial Dissemination, and HIF-1alpha Stabilization during Pneumonia. *MBio* 7. doi:10.1128/mBio.01397-16.

Holden, V.I., Lenio, S., Kuick, R., Ramakrishnan, S.K., Shah, Y.M., Bachman, M.A., 2014. Bacterial siderophores that evade or overwhelm lipocalin 2 induce hypoxia inducible factor 1alpha and proinflammatory cytokine secretion in cultured respiratory epithelial cells. *Infect Immun* 82, 3826-3836.

Holmes, M.A., Paulsene, W., Jide, X., Ratledge, C., Strong, R.K., 2005. Siderocalin (Lcn 2) also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. *Structure* 13, 29-41.

Hopfner, M., Nitsche, R., Rohr, A., Harms, D., Schubert, S., Folsch, U.R., 2001. Yersinia enterocolitica infection with multiple liver abscesses uncovering a primary hemochromatosis. *Scand J Gastroenterol* 36, 220-224.

Horvat, J.C., Beagley, K.W., Wade, M.A., Preston, J.A., Hansbro, N.G., Hickey, D.K., Kaiko, G.E., Gibson, P.G., Foster, P.S., Hansbro, P.M., 2007. Neonatal chlamydial infection induces mixed T-cell responses that drive allergic airway disease. *Am J Respir Crit Care Med* 176, 556-564.

Horvat, J.C., Starkey, M.R., Kim, R.Y., Beagley, K.W., Preston, J.A., Gibson, P.G., Foster, P.S., Hansbro, P.M., 2010a. Chlamydial respiratory infection during allergen sensitization drives neutrophilic allergic airways disease. *J Immunol* 184, 4159-4169.

Horvat, J.C., Starkey, M.R., Kim, R.Y., Phipps, S., Gibson, P.G., Beagley, K.W., Foster, P.S., Hansbro, P.M., 2010b. Early-life chlamydial lung infection enhances allergic airways disease through age-dependent differences in immunopathology. *J Allergy Clin Immunol* 125, 617-625, 625 e611-625 e616.

Hsu, A.C., Parsons, K., Moheimani, F., Knight, D.A., Hansbro, P.M., Fujita, T., Wark, P.A., 2016. Impaired Antiviral Stress Granule and IFN-beta Enhanceosome Formation Enhances Susceptibility to Influenza Infection in Chronic Obstructive Pulmonary Disease Epithelium. *Am J Respir Cell Mol Biol* 55, 117-127.

Hsu, A.C., See, H.V., Hansbro, P.M., Wark, P.A., 2012. Innate immunity to influenza in chronic airways diseases. *Respirology* 17, 1166-1175.

Hsu, A.C., Starkey, M.R., Hanish, I., Parsons, K., Haw, T.J., Howland, L.J., Barr, I., Mahony, J.B., Foster, P.S., Knight, D.A., Wark, P.A., Hansbro, P.M., 2015. Targeting PI3K-p110alpha Suppresses Influenza Virus Infection in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 191, 1012-1023.

Huang, C., Xie, M., He, X., Gao, H., 2013. Activity of sputum p38 MAPK is correlated with airway inflammation and reduced FEV1 in COPD patients. *Med Sci Monit* 19, 1229-1235.

Hunter, R.L., Bennett, B., Towns, M., Vogler, W.R., 1984. Transferrin in disease II: defects in the regulation of transferrin saturation with iron contribute to susceptibility to infection. *Am J Clin Pathol* 81, 748-753.

Hwang, S.A., Kruzell, M.L., Actor, J.K., 2014. Immunomodulatory effects of recombinant lactoferrin during MRSA infection. *Int Immunopharmacol* 20, 157-163.

Hwang, S.A., Wilk, K., Kruzel, M.L., Actor, J.K., 2009. A novel recombinant human lactoferrin augments the BCG vaccine and protects alveolar integrity upon infection with *Mycobacterium tuberculosis* in mice. *Vaccine* 27, 3026-3034.

Jabado, N., Jankowski, A., Dougaparsad, S., Picard, V., Grinstein, S., Gros, P., 2000. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. *J Exp Med* 192, 1237-1248.

Jenkins, Z.A., Hagar, W., Bowlus, C.L., Johansson, H.E., Harmatz, P., Vichinsky, E.P., Theil, E.C., 2007. Iron homeostasis during transfusional iron overload in beta-thalassemia and sickle cell disease: changes in iron regulatory protein, hepcidin, and ferritin expression. *Pediatr Hematol Oncol* 24, 237-243.

Johnson, E.E., Sandgren, A., Cherayil, B.J., Murray, M., Wessling-Resnick, M., 2010. Role of ferroportin in macrophage-mediated immunity. *Infect Immun* 78, 5099-5106.

Jones, B., Donovan, C., Liu, G., Gomez, H.M., Chimankar, V., Harrison, C.L., Wiegman, C.H., Adcock, I.M., Knight, D.A., Hirota, J.A., Hansbro, P.M., 2016. Animal models of COPD: What do they tell us? *Respirology* doi:10.1111/resp.12908.

Jurado, R.L., 1997. Iron, infections, and anemia of inflammation. *Clin Infect Dis* 25, 888-895.

Kato, J., Kobune, M., Ohkubo, S., Fujikawa, K., Tanaka, M., Takimoto, R., Takada, K., Takahari, D., Kawano, Y., Kohgo, Y., Niitsu, Y., 2007. Iron/IRP-1-dependent regulation of mRNA expression for transferrin receptor, DMT1 and ferritin during human erythroid differentiation. *Exp Hematol* 35, 879-887.

Keely, S., Talley, N.J., Hansbro, P.M., 2012. Pulmonary-intestinal cross-talk in mucosal inflammatory disease. *Mucosal Immunol* 5, 7-18.

Kemna, E., Pickkers, P., Nemeth, E., van der Hoeven, H., Swinkels, D., 2005. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood* 106, 1864-1866.

Kessler, M., Hoen, B., Mayeux, D., Hestin, D., Fontenaille, C., 1993. Bacteremia in patients on chronic hemodialysis. A multicenter prospective survey. *Nephron* 64, 95-100.

Khan, F.A., Fisher, M.A., Khakoo, R.A., 2007. Association of hemochromatosis with infectious diseases: expanding spectrum. *Int J Infect Dis* 11, 482-487.

Kim, K.H., Maldonado, F., Ryu, J.H., Eiken, P.W., Hartman, T.E., Bartholmai, B.J., Decker, P.A., Yi, E.S., 2010. Iron deposition and increased alveolar septal capillary density in nonfibrotic lung tissue are associated with pulmonary hypertension in idiopathic pulmonary fibrosis. *Respir Res* 11, 37.

Kim, R.Y., Horvat, J.C., Pinkerton, J.W., Starkey, M.R., Essilfie, A.T., Mayall, J.R., Nair, P.M., Hansbro, N.G., Jones, B., Haw, T.J., Sunkara, K.P., Nguyen, T.H., Jarnicki, A.G., Keely, S., Mattes, J., Adcock, I.M., Foster, P.S., Hansbro, P.M., 2016. MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase-mediated suppression of histone deacetylase 2. *J Allergy Clin Immunol*.

Kim, R.Y., Pinkerton, J.W., Gibson, P.G., Cooper, M.A., Horvat, J.C., Hansbro, P.M., 2015. Inflammasomes in COPD and neutrophilic asthma. *Thorax* 70, 1199-1201.

Kim, W.J., Wood, A.M., Barker, A.F., Brantly, M.L., Campbell, E.J., Eden, E., McElvaney, G., Rennard, S.I., Sandhaus, R.A., Stocks, J.M., Stoller, J.K., Strange, C., Turino, G., Silverman, E.K., Stockley, R.A., Demeo, D.L., 2012. Association of IREB2 and CHRNA3 polymorphisms with airflow obstruction in severe alpha-1 antitrypsin deficiency. *Respir Res* 13, 16.

King, P.T., Sharma, R., O'Sullivan, K., Selemidis, S., Lim, S., Radhakrishna, N., Lo, C., Prasad, J., Callaghan, J., McLaughlin, P., Farmer, M., Steinfert, D., Jennings, B., Ngui, J., Broughton, B.R., et al., 2015. Nontypeable *Haemophilus influenzae* induces sustained lung oxidative stress and protease expression. *PLoS One* 10, e0120371.

Knutson, M.D., Oukka, M., Koss, L.M., Aydemir, F., Wessling-Resnick, M., 2005. Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. *Proc Natl Acad Sci U S A* 102, 1324-1328.

Konings, A.F., Martin, L.W., Sharples, K.J., Roddam, L.F., Latham, R., Reid, D.W., Lamont, I.L., 2013. *Pseudomonas aeruginosa* uses multiple pathways to acquire iron during chronic infection in cystic fibrosis lungs. *Infect Immun* 81, 2697-2704.

Krause, A., Neitz, S., Magert, H.J., Schulz, A., Forssmann, W.G., Schulz-Knappe, P., Adermann, K., 2000. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett* 480, 147-150.

Kruzel, M.L., Bacsı, A., Choudhury, B., Sur, S., Boldogh, I., 2006. Lactoferrin decreases pollen antigen-induced allergic airway inflammation in a murine model of asthma. *Immunology* 119, 159-166.

Kukulj, S., Jaganjac, M., Boranic, M., Krizanac, S., Santic, Z., Poljak-Blazi, M., 2010. Altered iron metabolism, inflammation, transferrin receptors, and ferritin expression in non-small-cell lung cancer. *Med Oncol* 27, 268-277.

Laftah, A.H., Ramesh, B., Simpson, R.J., Solanky, N., Bahram, S., Schumann, K., Debnam, E.S., Srail, S.K., 2004. Effect of hepcidin on intestinal iron absorption in mice. *Blood* 103, 3940-3944.

Lagan, A.L., Quinlan, G.J., Mumby, S., Melley, D.D., Goldstraw, P., Bellingan, G.J., Hill, M.R., Briggs, D., Pantelidis, P., du Bois, R.M., Welsh, K.I., Evans, T.W., 2008. Variation in iron homeostasis genes between patients with ARDS and healthy control subjects. *Chest* 133, 1302-1311.

Lambe, T., Simpson, R.J., Dawson, S., Bouriez-Jones, T., Crockford, T.L., Lephherd, M., Latunde-Dada, G.O., Robinson, H., Raja, K.B., Campagna, D.R., Villarreal, G., Jr., Ellory, J.C., Goodnow, C.C., Fleming, M.D., McKie, A.T., Cornall, R.J., 2009. Identification of a Steap3 endosomal targeting motif essential for normal iron metabolism. *Blood* 113, 1805-1808.

Lamont, I.L., Konings, A.F., Reid, D.W., 2009. Iron acquisition by *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis. *Biometals* 22, 53-60.

Lawlor, M.S., O'Connor, C., Miller, V.L., 2007. Yersiniabactin is a virulence factor for *Klebsiella pneumoniae* during pulmonary infection. *Infect Immun* 75, 1463-1472.

Lee, P., Peng, H., Gelbart, T., Wang, L., Beutler, E., 2005. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proc Natl Acad Sci U S A* 102, 1906-1910.

Levy, J.E., Montross, L.K., Cohen, D.E., Fleming, M.D., Andrews, N.C., 1999. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. *Blood* 94, 9-11.

Lin, L., Valore, E.V., Nemeth, E., Goodnough, J.B., Gabayan, V., Ganz, T., 2007. Iron transferrin regulates hepcidin synthesis in primary hepatocyte culture through hemojuvelin and BMP2/4. *Blood* 110, 2182-2189.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., et al., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380, 2095-2128.

Ludwiczek, S., Aigner, E., Theurl, I., Weiss, G., 2003. Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood* 101, 4148-4154.

Luo, M., Fadeev, E.A., Groves, J.T., 2005. Mycobactin-mediated iron acquisition within macrophages. *Nat Chem Biol* 1, 149-153.

Maazi, H., Shirinbak, S., Bloksma, N., Nawijn, M.C., van Oosterhout, A.J., 2011. Iron administration reduces airway hyperreactivity and eosinophilia in a mouse model of allergic asthma. *Clin Exp Immunol* 166, 80-86.

MacKenzie, E.L., Iwasaki, K., Tsuji, Y., 2008. Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10, 997-1030.

Maffettone, C., Chen, G., Drozdov, I., Ouzounis, C., Pantopoulos, K., 2010. Tumorigenic properties of iron regulatory protein 2 (IRP2) mediated by its specific 73-amino acids insert. *PLoS One* 5, e10163.

Marshall, L.J., Oguejiofor, W., Price, R., Shur, J., 2016. Investigation of the enhanced antimicrobial activity of combination dry powder inhaler formulations of lactoferrin. *Int J Pharm* 514, 399-406.

Marsland, B.J., Trompette, A., Gollwitzer, E.S., 2015. The Gut-Lung Axis in Respiratory Disease. *Ann Am Thorac Soc* 12 Suppl 2, S150-156.

Martineau, A.R., Newton, S.M., Wilkinson, K.A., Kampmann, B., Hall, B.M., Nawroly, N., Packe, G.E., Davidson, R.N., Griffiths, C.J., Wilkinson, R.J., 2007. Neutrophil-mediated innate immune resistance to mycobacteria. *J Clin Invest* 117, 1988-1994.

Mason, W.J., Skaar, E.P., 2009. Assessing the contribution of heme-iron acquisition to *Staphylococcus aureus* pneumonia using computed tomography. *PLoS One* 4, e6668.

Mazmanian, S.K., Skaar, E.P., Gaspar, A.H., Humayun, M., Gornicki, P., Jelenska, J., Joachmiak, A., Missiakas, D.M., Schneewind, O., 2003. Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* 299, 906-909.

McKie, A.T., Barrow, D., Latunde-Dada, G.O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T.J., Raja, K.B., Shirali, S., Hediger, M.A., Farzaneh, F., Simpson, R.J., 2001. An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 291, 1755-1759.

Meyron-Holtz, E.G., Cohen, L.A., Fahoum, L., Haimovich, Y., Lifshitz, L., Magid-Gold, I., Stuemler, T., Truman-Rosentsvit, M., 2014. Ferritin polarization and iron transport across monolayer epithelial barriers in mammals. *Front Pharmacol* 5, 194.

Miller, M.J., Walz, A.J., Zhu, H., Wu, C., Moraski, G., Mollmann, U., Tristani, E.M., Crumbliss, A.L., Ferdig, M.T., Checkley, L., Edwards, R.L., Boshoff, H.I., 2011. Design, synthesis, and study of a mycobactin-artemisinin conjugate that has selective and potent activity against tuberculosis and malaria. *J Am Chem Soc* 133, 2076-2079.

Milman N, P.L., 2002. The serum ferritin concentration is a significant prognostic indicator of survival in primary lung cancer. *Oncol Rep* 9, 193-198.

Minandri, F., Imperi, F., Frangipani, E., Bonchi, C., Visaggio, D., Facchini, M., Pasquali, P., Bragonzi, A., Visca, P., 2016. Role of Iron Uptake Systems in *Pseudomonas aeruginosa* Virulence and Airway Infection. *Infect Immun* 84, 2324-2335.

Mitchell, T.J., 2000. Virulence factors and the pathogenesis of disease caused by *Streptococcus pneumoniae*. *Res Microbiol* 151, 413-419.

Muckenthaler, M.U., Galy, B., Hentze, M.W., 2008. Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. *Annu Rev Nutr* 28, 197-213.

Mulero, V., Searle, S., Blackwell, J.M., Brock, J.H., 2002. Solute carrier 11a1 (Slc11a1; formerly Nramp1) regulates metabolism and release of iron acquired by phagocytic, but not transferrin-receptor-mediated, iron uptake. *Biochem J* 363, 89-94.

Muryoi, N., Tiedemann, M.T., Pluym, M., Cheung, J., Heinrichs, D.E., Stillman, M.J., 2008. Demonstration of the iron-regulated surface determinant (Isd) heme transfer pathway in *Staphylococcus aureus*. *J Biol Chem* 283, 28125-28136.

Nairz, M., Fritsche, G., Brunner, P., Talasz, H., Hantke, K., Weiss, G., 2008. Interferon-gamma limits the availability of iron for intramacrophage *Salmonella typhimurium*. *Eur J Immunol* 38, 1923-1936.

Nairz, M., Haschka, D., Demetz, E., Weiss, G., 2014. Iron at the interface of immunity and infection. *Front Pharmacol* 5, 152.

Nairz, M., Theurl, I., Ludwiczek, S., Theurl, M., Mair, S.M., Fritsche, G., Weiss, G., 2007. The coordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular *Salmonella typhimurium*. *Cell Microbiol* 9, 2126-2140.

Nairz, M., Theurl, I., Schroll, A., Theurl, M., Fritsche, G., Lindner, E., Seifert, M., Crouch, M.L., Hantke, K., Akira, S., Fang, F.C., Weiss, G., 2009. Absence of functional Hfe protects mice from invasive *Salmonella enterica* serovar Typhimurium infection via induction of lipocalin-2. *Blood* 114, 3642-3651.

Nelson, A.L., Ratner, A.J., Barasch, J., Weiser, J.N., 2007. Interleukin-8 secretion in response to aferric enterobactin is potentiated by siderocalin. *Infect Immun* 75, 3160-3168.

Nelson, M.E., O'Brien-Ladner, A.R., Wesselius, L.J., 1996. Regional variation in iron and iron-binding proteins within the lungs of smokers. *Am J Respir Crit Care Med* 153, 1353-1358.

Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Ganz, T., Kaplan, J., 2004. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 306, 2090-2093.

Nguyen, N.B., Callaghan, K.D., Ghio, A.J., Haile, D.J., Yang, F., 2006. Heparin expression and iron transport in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 291, L417-425.

Nicolas, G., Bennoun, M., Porteu, A., Mativet, S., Beaumont, C., Grandchamp, B., Sirito, M., Sawadogo, M., Kahn, A., Vaulont, S., 2002a. Severe iron deficiency anemia in transgenic mice expressing liver heparin. *Proc Natl Acad Sci U S A* 99, 4596-4601.

Nicolas, G., Chauvet, C., Viatte, L., Danan, J.L., Bigard, X., Devaux, I., Beaumont, C., Kahn, A., Vaulont, S., 2002b. The gene encoding the iron regulatory peptide heparin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 110, 1037-1044.

O'Brien-Ladner, A.R., Nelson, S.R., Murphy, W.J., Blumer, B.M., Wesselius, L.J., 2000. Iron is a regulatory component of human IL-1 β production. Support for regional variability in the lung. *Am J Respir Cell Mol Biol* 23, 112-119.

O'Sullivan, B.P., Freedman, S.D., 2009. Cystic fibrosis. *Lancet* 373, 1891-1904.

Olanemi, O., Schlesinger, L.S., Ahmed, A., Britigan, B.E., 2002. Intraphagosomal Mycobacterium tuberculosis acquires iron from both extracellular transferrin and intracellular iron pools. Impact of interferon-gamma and hemochromatosis. *J Biol Chem* 277, 49727-49734.

Olson, A.L., Swigris, J.J., 2012. Idiopathic pulmonary fibrosis: diagnosis and epidemiology. *Clin Chest Med* 33, 41-50.

Pantopoulos, K., Porwal, S.K., Tartakoff, A., Devireddy, L., 2012. Mechanisms of mammalian iron homeostasis. *Biochemistry* 51, 5705-5724.

Parrow, N.L., Fleming, R.E., 2014. Bone morphogenetic proteins as regulators of iron metabolism. *Annu Rev Nutr* 34, 77-94.

Parrow, N.L., Fleming, R.E., Minnick, M.F., 2013. Sequestration and scavenging of iron in infection. *Infect Immun* 81, 3503-3514.

Paul, V.D., Lill, R., 2015. Biogenesis of cytosolic and nuclear iron-sulfur proteins and their role in genome stability. *Biochim Biophys Acta* 1853, 1528-1539.

Payne, S.M., 1993. Iron acquisition in microbial pathogenesis. *Trends Microbiol* 1, 66-69.

Peters, A.L., van Hezel, M.E., Juffermans, N.P., Vlaar, A.P., 2015. Pathogenesis of non-antibody mediated transfusion-related acute lung injury from bench to bedside. *Blood Rev* 29, 51-61.

Peyssonnaud, C., Zinkernagel, A.S., Datta, V., Lauth, X., Johnson, R.S., Nizet, V., 2006. TLR4-dependent heparin expression by myeloid cells in response to bacterial pathogens. *Blood* 107, 3727-3732.

Philippot, Q., Deslee, G., Adair-Kirk, T.L., Woods, J.C., Byers, D., Conradi, S., Dury, S., Perotin, J.M., Lebargy, F., Cassan, C., Le Naour, R., Holtzman, M.J., Pierce, R.A., 2014. Increased iron sequestration in alveolar macrophages in chronic obstructive pulmonary disease. *PLoS One* 9, e96285.

Philpott, C.C., Ryu, M.S., 2014. Special delivery: distributing iron in the cytosol of mammalian cells. *Front Pharmacol* 5, 173.

Pidcock, K.A., Wooten, J.A., Daley, B.A., Stull, T.L., 1988. Iron acquisition by Haemophilus influenzae. *Infect Immun* 56, 721-725.

Pietrantonio, A., Dofrelli, E., Tinari, A., Ammendolia, M.G., Puzelli, S., Fabiani, C., Donatelli, I., Superti, F., 2010. Bovine lactoferrin inhibits influenza A virus induced programmed cell death in vitro. *Biometals* 23, 465-475.

Pigeon, C., Ilyin, G., Courselaud, B., Leroyer, P., Turlin, B., Brissot, P., Loreal, O., 2001. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide heparin, is overexpressed during iron overload. *J Biol Chem* 276, 7811-7819.

Pillai, S.G., Ge, D., Zhu, G., Kong, X., Shianna, K.V., Need, A.C., Feng, S., Hersh, C.P., Bakke, P., Gulsvik, A., Ruppert, A., Lodrup Carlsen, K.C., Roses, A., Anderson, W., Rennard, S.I., Lomas, D.A., Silverman, E.K., Goldstein, D.B., Investigators, I., 2009. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 5, e1000421.

Pond, M.N., Morton, A.M., Conway, S.P., 1996. Functional iron deficiency in adults with cystic fibrosis. *Respir Med* 90, 409-413.

Powell, L.W., Seckington, R.C., Deugnier, Y., 2016. Haemochromatosis. *Lancet* 388, 706-716.

Puxeddu, E., Comandini, A., Cavalli, F., Pezzuto, G., D'Ambrosio, C., Senis, L., Paci, M., Curradi, G., Sergiacomi, G.L., Saltini, C., 2014. Iron laden macrophages in idiopathic pulmonary fibrosis: the telltale of occult alveolar hemorrhage? *Pulm Pharmacol Ther* 28, 35-40.

Rabe, K.F., Hurd, S., Anzueto, A., Barnes, P.J., Buist, S.A., Calverley, P., Fukuchi, Y., Jenkins, C., Rodriguez-Roisin, R., van Weel, C., Zielinski, J., Global Initiative for Chronic Obstructive Lung, D., 2007. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 176, 532-555.

Raghu, G., Collard, H.R., Egan, J.J., Martinez, F.J., Behr, J., Brown, K.K., Colby, T.V., Cordier, J.F., Flaherty, K.R., Lasky, J.A., Lynch, D.A., Ryu, J.H., Swigris, J.J., Wells, A.U., Ancochea, J., et al., 2011. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 183, 788-824.

Ramakrishnan, K., Borade, A., 2010. Anemia as a risk factor for childhood asthma. *Lung India* 27, 51-53.

Reid, D., Snell, G., Ward, C., Krishnaswamy, R., Ward, R., Zheng, L., Williams, T., Walters, H., 2001. Iron overload and nitric oxide-derived oxidative stress following lung transplantation. *J Heart Lung Transplant* 20, 840-849.

Reid, D.W., Carroll, V., O'May, C., Champion, A., Kirov, S.M., 2007. Increased airway iron as a potential factor in the persistence of *Pseudomonas aeruginosa* infection in cystic fibrosis. *Eur Respir J* 30, 286-292.

Reid, D.W., Lam, Q.T., Schneider, H., Walters, E.H., 2004. Airway iron and iron-regulatory cytokines in cystic fibrosis. *Eur Respir J* 24, 286-291.

Reid, D.W., Withers, N.J., Francis, L., Wilson, J.W., Kotsimbos, T.C., 2002. Iron deficiency in cystic fibrosis: relationship to lung disease severity and chronic *Pseudomonas aeruginosa* infection. *Chest* 121, 48-54.

Rivera, S., Nemeth, E., Gabayan, V., Lopez, M.A., Farshidi, D., Ganz, T., 2005. Synthetic hepcidin causes rapid dose-dependent hypoferrremia and is concentrated in ferroportin-containing organs. *Blood* 106, 2196-2199.

Romero-Espejel, M.E., Gonzalez-Lopez, M.A., Olivares-Trejo Jde, J., 2013. *Streptococcus pneumoniae* requires iron for its viability and expresses two membrane proteins that bind haemoglobin and haem. *Metallomics* 5, 384-389.

Rosen, S.H., Castleman, B., Liebow, A.A., 1958. Pulmonary alveolar proteinosis. *N Engl J Med* 258, 1123-1142.

Rouault, T.A., Tong, W.H., 2008. Iron-sulfur cluster biogenesis and human disease. *Trends Genet* 24, 398-407.

Ruiz-Morales, J.M., Dorantes-Heredia, R., Arrieta, O., Chavez-Tapia, N.C., Motola-Kuba, D., 2015. Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP-9) prognostic value in lung adenocarcinoma. *Tumour Biol* 36, 3601-3610.

Sandmeier, P., Speich, R., Grebski, E., Vogt, P., Russi, E.W., Weder, W., Boehler, A., 2005. Iron accumulation in lung allografts is associated with acute rejection but not with adverse outcome. *Chest* 128, 1379-1384.

Sanguuolo, F., Puxeddu, E., Pezzuto, G., Cavalli, F., Longo, G., Comandini, A., Di Pierro, D., Pallante, M., Sergiacomi, G., Simonetti, G., Zompatori, M., Orlandi, A., Magrini, A., Amicosante, M., Mariani, F., Losi, M., Fraboni, D., Bisetti, A., Saltini, C., 2015. HFE gene variants and iron-induced oxygen radical generation in idiopathic pulmonary fibrosis. *Eur Respir J* 45, 483-490.

Sapkota, B.R., Hijikata, M., Matsushita, I., Tanaka, G., Ieki, R., Kobayashi, N., Toyota, E., Nagai, H., Kurashima, A., Tokunaga, K., Keicho, N., 2012. Association of SLC11A1 (NRAMP1) polymorphisms with pulmonary *Mycobacterium avium* complex infection. *Hum Immunol* 73, 529-536.

Schaible, U.E., Collins, H.L., Priem, F., Kaufmann, S.H., 2002. Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. *J Exp Med* 196, 1507-1513.

Schmidt, P.J., Toran, P.T., Giannetti, A.M., Bjorkman, P.J., Andrews, N.C., 2008. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. *Cell Metab* 7, 205-214.

Schryvers, A.B., Gray-Owen, S., 1992. Iron acquisition in *Haemophilus influenzae*: receptors for human transferrin. *J Infect Dis* 165 Suppl 1, S103-104.

Schryvers, A.B., Morris, L.J., 1988. Identification and characterization of the transferrin receptor from *Neisseria meningitidis*. *Mol Microbiol* 2, 281-288.

Sharkey, R.A., Donnelly, S.C., Connelly, K.G., Robertson, C.E., Haslett, C., Repine, J.E., 1999. Initial serum ferritin levels in patients with multiple trauma and the subsequent development of acute respiratory distress syndrome. *Am J Respir Crit Care Med* 159, 1506-1509.

Shi, H.B., Li, X.D., Jiang, J.T., Zhao, W.Q., Ji, M., Wu, C.P., 2014. Serum ferritin is elevated in advanced non-small cell lung cancer patients and is associated with efficacy of platinum-based chemotherapy. *J Cancer Res Ther* 10, 681-685.

Shiiba, M., Saito, K., Fushimi, K., Ishigami, T., Shinozuka, K., Nakashima, D., Kouzu, Y., Koike, H., Kasamatsu, A., Sakamoto, Y., Ogawara, K., Uzawa, K., Takiguchi, Y., Tanzawa, H., 2013. Lipocalin-2 is associated with radioresistance in oral cancer and lung cancer cells. *Int J Oncol* 42, 1197-1204.

Shimizu, Y., Matsuzaki, S., Dobashi, K., Yanagitani, N., Satoh, T., Koka, M., Yokoyama, A., Ohkubo, T., Ishii, Y., Kamiya, T., Mori, M., 2011. Elemental analysis of lung tissue particles and intracellular iron content of alveolar macrophages in pulmonary alveolar proteinosis. *Respir Res* 12, 88.

Shin, K., Wakabayashi, H., Yamauchi, K., Teraguchi, S., Tamura, Y., Kurokawa, M., Shiraki, K., 2005. Effects of orally administered bovine lactoferrin and lactoperoxidase on influenza virus infection in mice. *J Med Microbiol* 54, 717-723.

Simpson, J.L., Baines, K.J., Horvat, J.C., Essilfie, A.T., Brown, A.C., Tooze, M., McDonald, V.M., Gibson, P.G., Hansbro, P.M., 2016. COPD is characterized by increased detection of *Haemophilus influenzae*, *Streptococcus pneumoniae* and a deficiency of *Bacillus* species. *Respirology* 21, 697-704.

Simpson, J.L., Powell, H., Baines, K.J., Milne, D., Coxson, H.O., Hansbro, P.M., Gibson, P.G., 2014. The effect of azithromycin in adults with stable neutrophilic COPD: a double blind randomised, placebo controlled trial. *PLoS One* 9, e105609.

Singanayagam, A., Glanville, N., Walton, R.P., Aniscenko, J., Pearson, R.M., Pinkerton, J.W., Horvat, J.C., Hansbro, P.M., Bartlett, N.W., Johnston, S.L., 2015. A short-term mouse model that reproduces the immunopathological features of rhinovirus-induced exacerbation of COPD. *Clin Sci (Lond)* 129, 245-258.

Singh, P.K., Parsek, M.R., Greenberg, E.P., Welsh, M.J., 2002. A component of innate immunity prevents bacterial biofilm development. *Nature* 417, 552-555.

Skaar, E.P., 2010. The battle for iron between bacterial pathogens and their vertebrate hosts. *PLoS Pathog* 6, e1000949.

Skaar, E.P., Gaspar, A.H., Schneewind, O., 2004a. IsdG and IsdI, heme-degrading enzymes in the cytoplasm of *Staphylococcus aureus*. *J Biol Chem* 279, 436-443.

Skaar, E.P., Humayun, M., Bae, T., DeBord, K.L., Schneewind, O., 2004b. Iron-source preference of *Staphylococcus aureus* infections. *Science* 305, 1626-1628.

Smith, C.L., Arvedson, T.L., Cooke, K.S., Dickmann, L.J., Forte, C., Li, H., Merriam, K.L., Perry, V.K., Tran, L., Rottman, J.B., Maxwell, J.R., 2013a. IL-22 regulates iron availability in vivo through the induction of hepcidin. *J Immunol* 191, 1845-1855.

Smith, D.J., Lamont, I.L., Anderson, G.J., Reid, D.W., 2013b. Targeting iron uptake to control *Pseudomonas aeruginosa* infections in cystic fibrosis. *Eur Respir J* 42, 1723-1736.

Soares, M.P., Weiss, G., 2015. The Iron age of host-microbe interactions. *EMBO Rep* 16, 1482-1500.

Soe-Lin, S., Apte, S.S., Andriopoulos, B., Jr., Andrews, M.C., Schranzhofer, M., Kahawita, T., Garcia-Santos, D., Ponka, P., 2009. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. *Proc Natl Acad Sci U S A* 106, 5960-5965.

Starkey, M.R., Essilfie, A.T., Horvat, J.C., Kim, R.Y., Nguyen, D.H., Beagley, K.W., Mattes, J., Foster, P.S., Hansbro, P.M., 2013a. Constitutive production of IL-13 promotes early-life Chlamydia respiratory infection and allergic airway disease. *Mucosal Immunol* 6, 569-579.

Starkey, M.R., Jarnicki, A.G., Essilfie, A.T., Gellatly, S.L., Kim, R.Y., Brown, A.C., Foster, P.S., Horvat, J.C., Hansbro, P.M., 2013b. Murine models of infectious exacerbations of airway inflammation. *Curr Opin Pharmacol* 13, 337-344.

Starkey, M.R., Kim, R.Y., Beckett, E.L., Schilter, H.C., Shim, D., Essilfie, A.T., Nguyen, D.H., Beagley, K.W., Mattes, J., Mackay, C.R., Horvat, J.C., Hansbro, P.M., 2012. Chlamydia muridarum lung infection in infants alters hematopoietic cells to promote allergic airway disease in mice. *PLoS One* 7, e42588.

Starkey, M.R., Nguyen, D.H., Brown, A.C., Essilfie, A.T., Kim, R.Y., Yagita, H., Horvat, J.C., Hansbro, P.M., 2016. Programmed Death Ligand 1 Promotes Early-Life Chlamydia Respiratory Infection-Induced Severe Allergic Airway Disease. *Am J Respir Cell Mol Biol* 54, 493-503.

Starkey, M.R., Nguyen, D.H., Essilfie, A.T., Kim, R.Y., Hatchwell, L.M., Collison, A.M., Yagita, H., Foster, P.S., Horvat, J.C., Mattes, J., Hansbro, P.M., 2014. Tumor necrosis factor-related apoptosis-inducing ligand translates neonatal respiratory infection into chronic lung disease. *Mucosal Immunol* 7, 478-488.

Stites, S.W., Plautz, M.W., Bailey, K., O'Brien-Ladner, A.R., Wesselius, L.J., 1999. Increased concentrations of iron and isoferritins in the lower respiratory tract of patients with stable cystic fibrosis. *Am J Respir Crit Care Med* 160, 796-801.

Stites, S.W., Walters, B., O'Brien-Ladner, A.R., Bailey, K., Wesselius, L.J., 1998. Increased iron and ferritin content of sputum from patients with cystic fibrosis or chronic bronchitis. *Chest* 114, 814-819.

Sutak, R., Lesuisse, E., Tachezy, J., Richardson, D.R., 2008. Crusade for iron: iron uptake in unicellular eukaryotes and its significance for virulence. *Trends Microbiol* 16, 261-268.

Tai, S.S., Lee, C.J., Winter, R.E., 1993. Hemin utilization is related to virulence of *Streptococcus pneumoniae*. *Infect Immun* 61, 5401-5405.

Tanaka, G., Shojima, J., Matsushita, I., Nagai, H., Kurashima, A., Nakata, K., Toyota, E., Kobayashi, N., Kudo, K., Keicho, N., 2007. Pulmonary Mycobacterium avium complex infection: association with NRAMP1 polymorphisms. *Eur Respir J* 30, 90-96.

Tandara, L., Grubisic, T.Z., Ivan, G., Jurisic, Z., Tandara, M., Gugo, K., Mladinov, S., Salamunic, I., 2015. Systemic inflammation up-regulates serum hepcidin in exacerbations and stable chronic obstructive pulmonary disease. *Clin Biochem* 48, 1252-1257.

Tay, H.L., Kaiko, G.E., Plank, M., Li, J., Maltby, S., Essilfie, A.T., Jarnicki, A., Yang, M., Mattes, J., Hansbro, P.M., Foster, P.S., 2015. Correction: Antagonism of miR-328 Increases the Antimicrobial Function of Macrophages and Neutrophils and Rapid Clearance of Non-typeable Haemophilus Influenzae (NTHi) from Infected Lung. *PLoS Pathog* 11, e1004956.

Theurl, I., Theurl, M., Seifert, M., Mair, S., Nairz, M., Rumpold, H., Zoller, H., Bellmann-Weiler, R., Niederegger, H., Talasz, H., Weiss, G., 2008. Autocrine formation of hepcidin induces iron retention in human monocytes. *Blood* 111, 2392-2399.

Thompson, A.B., Bohling, T., Heires, A., Linder, J., Rennard, S.I., 1991. Lower respiratory tract iron burden is increased in association with cigarette smoking. *J Lab Clin Med* 117, 493-499.

Thun, M.J., Hannan, L.M., Adams-Campbell, L.L., Boffetta, P., Buring, J.E., Feskanich, D., Flanders, W.D., Jee, S.H., Katanoda, K., Kolonel, L.N., Lee, I.M., Marugame, T., Palmer, J.R., Riboli, E., Sobue, T., Avila-Tang, E., Wilkens, L.R., Samet, J.M., 2008. Lung cancer occurrence in never-smokers: an analysis of 13 cohorts and 22 cancer registry studies. *PLoS Med* 5, e185.

Traore, H.N., Meyer, D., 2007. Necrosis of host cells and survival of pathogens following iron overload in an in vitro model of co-infection with human immunodeficiency virus (HIV) and Mycobacterium tuberculosis. *Int J Antimicrob Agents* 29, 465-470.

Turi, J.L., Yang, F., Garrick, M.D., Piantadosi, C.A., Ghio, A.J., 2004. The iron cycle and oxidative stress in the lung. *Free Radic Biol Med* 36, 850-857.

Uijterschout, L., Nuijsink, M., Hendriks, D., Vos, R., Brus, F., 2014. Iron deficiency occurs frequently in children with cystic fibrosis. *Pediatr Pulmonol* 49, 458-462.

Upton, R.L., Chen, Y., Mumby, S., Gutteridge, J.M., Anning, P.B., Nicholson, A.G., Evans, T.W., Quinlan, G.J., 2003. Variable tissue expression of transferrin receptors: relevance to acute respiratory distress syndrome. *Eur Respir J* 22, 335-341.

Van Zandt, K.E., Sow, F.B., Florence, W.C., Zwilling, B.S., Satoskar, A.R., Schlesinger, L.S., Lafuse, W.P., 2008. The iron export protein ferroportin 1 is differentially expressed in mouse macrophage populations and is present in the mycobacterial-containing phagosome. *J Leukoc Biol* 84, 689-700.

Vasil, M.L., Ochsner, U.A., 1999. The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. *Mol Microbiol* 34, 399-413.

Velliyagounder, K., Alsaedi, W., Alabdulmohsen, W., Markowitz, K., Fine, D.H., 2015. Oral lactoferrin protects against experimental candidiasis in mice. *J Appl Microbiol* 118, 212-221.

Vidal, S., Gros, P., Skamene, E., 1995a. Natural resistance to infection with intracellular parasites: molecular genetics identifies Nramp1 as the Bcg/Ity/Lsh locus. *J Leukoc Biol* 58, 382-390.

Vidal, S., Tremblay, M.L., Govoni, G., Gauthier, S., Sebastiani, G., Malo, D., Skamene, E., Olivier, M., Jothy, S., Gros, P., 1995b. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. *J Exp Med* 182, 655-666.

Vlasic, Z., Dodig, S., Cepelak, I., Topic, R.Z., Zivcic, J., Nogalo, B., Turkalj, M., 2009. Iron and ferritin concentrations in exhaled breath condensate of children with asthma. *J Asthma* 46, 81-85.

Vogel, L., Geluk, F., Jansen, H., Dankert, J., van Alphen, L., 1997a. Human lactoferrin receptor activity in non-encapsulated *Haemophilus influenzae*. *FEMS Microbiol Lett* 156, 165-170.

Vogel, L., Schoonbrood, D., Geluk, F., Hoek, F., Bresser, P., Out, T., Jansen, H., Dankert, J., van Alphen, L., 1997b. Iron-binding proteins in sputum of chronic bronchitis patients with *Haemophilus influenzae* infections. *Eur Respir J* 10, 2327-2333.

Vostrejs, M., Moran, P.L., Seligman, P.A., 1988. Transferrin synthesis by small cell lung cancer cells acts as an autocrine regulator of cellular proliferation. *J Clin Invest* 82, 331-339.

Wakabayashi, H., Oda, H., Yamauchi, K., Abe, F., 2014. Lactoferrin for prevention of common viral infections. *J Infect Chemother* 20, 666-671.

Wanachiwanawin, W., 2000. Infections in E-beta thalassemia. *J Pediatr Hematol Oncol* 22, 581-587.

Wang B, Z.J., Song F, Tian M, Shi B, Jiang H, Xu W, Wang H, Zhou M, Pan X, Gu J, Yang S, Jiang L, Li Z, 2016. EGFR regulates iron homeostasis to promote cancer growth through redistribution of transferrin receptor 1. *Cancer Letters* 381, 331-340.

Wang, W., Li, J.J., Foster, P.S., Hansbro, P.M., Yang, M., 2010. Potential therapeutic targets for steroid-resistant asthma. *Curr Drug Targets* 11, 957-970.

Wang, X., Garrick, M.D., Yang, F., Dailey, L.A., Piantadosi, C.A., Ghio, A.J., 2005. TNF, IFN-gamma, and endotoxin increase expression of DMT1 in bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 289, L24-33.

Wang, X., Ghio, A.J., Yang, F., Dolan, K.G., Garrick, M.D., Piantadosi, C.A., 2002. Iron uptake and Nramp2/DMT1/DCT1 in human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 282, L987-995.

Ward, P.P., Uribe-Luna, S., Conneely, O.M., 2002. Lactoferrin and host defense. *Biochem Cell Biol* 80, 95-102.

Webb, D.C., Cripps, A.W., 1999. Immunization with recombinant transferrin binding protein B enhances clearance of nontypeable *Haemophilus influenzae* from the rat lung. *Infect Immun* 67, 2138-2144.

Weinberg, E.D., 2009. Iron availability and infection. *Biochim Biophys Acta* 1790, 600-605.

Weinstein, D.A., Roy, C.N., Fleming, M.D., Loda, M.F., Wolfsdorf, J.I., Andrews, N.C., 2002. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood* 100, 3776-3781.

Welsh, K.J., Hwang, S.A., Boyd, S., Kruzel, M.L., Hunter, R.L., Actor, J.K., 2011. Influence of oral lactoferrin on Mycobacterium tuberculosis induced immunopathology. *Tuberculosis (Edinb)* 91 Suppl 1, S105-113.

Wesselius, L.J., Nelson, M.E., Skikne, B.S., 1994. Increased release of ferritin and iron by iron-loaded alveolar macrophages in cigarette smokers. *Am J Respir Crit Care Med* 150, 690-695.

Wessling-Resnick, M., 2006. Iron imports. III. Transfer of iron from the mucosa into circulation. *Am J Physiol Gastrointest Liver Physiol* 290, G1-6.

WHO, 2011. World Health Statistics 2011. <http://www.who.int/whosis/whostat/2011/en/index.html>

WHO, 2014. World Cancer Report IARC Nonserial Publication, distributed by WHO Press.

WHO, March 2015. COPD fact sheet . <http://www.who.int/mediacentre/factsheets/fs315/en/>

Williams, K.J., 2014. Gammaherpesviruses and pulmonary fibrosis: evidence from humans, horses, and rodents. *Vet Pathol* 51, 372-384.

Wood, L.G., Simpson, J.L., Hansbro, P.M., Gibson, P.G., 2010. Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia. *Free Radic Res* 44, 146-154.

Wrighting, D.M., Andrews, N.C., 2006. Interleukin-6 induces hepcidin expression through STAT3. *Blood* 108, 3204-3209.

Wu, H., Santoni-Rugiu, E., Ralfkiaer, E., Porse, B.T., Moser, C., Høiby, N., Borregaard, N., Cowland, J.B., 2010. Lipocalin 2 is protective against E. coli pneumonia. *Respir Res* 11, 96.

Wu, Q., Jiang, D., Minor, M.N., Martin, R.J., Chu, H.W., 2011. In vivo function of airway epithelial TLR2 in host defense against bacterial infection. *Am J Physiol Lung Cell Mol Physiol* 300, L579-586.

Yamaji, S., Sharp, P., Ramesh, B., Srani, S.K., 2004. Inhibition of iron transport across human intestinal epithelial cells by hepcidin. *Blood* 104, 2178-2180.

Yang, F., Wang, X., Haile, D.J., Piantadosi, C.A., Ghio, A.J., 2002. Iron increases expression of iron-export protein MTP1 in lung cells. *Am J Physiol Lung Cell Mol Physiol* 283, L932-939.

Yang, M., Kumar, R.K., Hansbro, P.M., Foster, P.S., 2012. Emerging roles of pulmonary macrophages in driving the development of severe asthma. *J Leukoc Biol* 91, 557-569.

Yi, K., Sethi, S., Murphy, T.F., 1997. Human immune response to nontypeable Haemophilus influenzae in chronic bronchitis. *J Infect Dis* 176, 1247-1252.

Zagulski T, L.P., Zagulska A, Broniek S, Jarzabek Z., 1989. Lactoferrin can protect mice against a lethal dose of Escherichia coli in experimental infection in vivo. *Br J Exp Pathol* 70, 697-704.

Zhang, C., 2014. Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control. *Protein Cell* 5, 750-760.

Zhang, W., Shao, L., Weng, X., Hu, Z., Jin, A., Chen, S., Pang, M., Chen, Z.W., 2005. Variants of the natural resistance-associated macrophage protein 1 gene (NRAMP1) are associated with severe forms of pulmonary tuberculosis. *Clin Infect Dis* 40, 1232-1236.

Zhou, H., Yang, J., Li, D., Xiao, J., Wang, B., Wang, L., Ma, C., Xu, S., Ou, X., Feng, Y., 2012. Association of IREB2 and CHRNA3/5 polymorphisms with COPD and COPD-related phenotypes in a Chinese Han population. *J Hum Genet* 57, 738-746.

Zhou, X.Y., Tomatsu, S., Fleming, R.E., Parkkila, S., Waheed, A., Jiang, J., Fei, Y., Brunt, E.M., Ruddy, D.A., Prass, C.E., Schatzman, R.C., O'Neill, R., Britton, R.S., Bacon, B.R., Sly, W.S., 1998. HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proc Natl Acad Sci U S A* 95, 2492-2497.

Ziolkowska-Suchanek, I., Mosor, M., Gabryel, P., Grabicki, M., Zurawek, M., Fichna, M., Strauss, E., Batura-Gabryel, H., Dyszkiewicz, W., Nowak, J., 2015. Susceptibility loci in lung cancer and COPD: association of IREB2 and FAM13A with pulmonary diseases. *Sci Rep* 5, 13502.

Zwilling, B.S., Kuhn, D.E., Wikoff, L., Brown, D., Lafuse, W., 1999. Role of iron in Nramp1-mediated inhibition of mycobacterial growth. *Infect Immun* 67, 1386-1392.