Pathogenesis of the systemic inflammatory syndrome and acute lung injury: role of iron mobilization and decompartmentalization

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Lagan AL, Melley DD, Evans TW, Quinlan GJ. Pathogenesis of the systemic inflammatory syndrome and acute lung injury: role of iron mobilization and decompartmentalization. Am J Physiol Lung Cell Mol Physiol 294: L161–L174, 2008. First published November 30, 2007; doi:10.1152/ajplung.00169.2007.—Changes in iron homeostatic responses routinely accompany infectious or proinflammatory insults. The systemic inflammatory response syndrome (SIRS) and the development of acute lung injury (ALI) feature pronounced systemic and lung-specific alterations in iron/heme mobilization and decompartmentalization; such responses may be of pathological significance for both the onset and progression of acute inflammation. The potential for excessive iron-catalyzed oxidative stress, altered proinflammatory redox signaling, and provision of iron as a microbial growth factor represent obvious adverse aspects of altered iron handling. The release of hemoglobin during hemolytic disease or surgical procedures such as those utilizing cardiopulmonary bypass procedures further impacts on iron mobilization, turnover, and storage with associated implications. Genetic predisposition may ultimately determine the extent to which SIRS and related syndromes develop in response to such changes. The design of specific therapeutic interventions based on endogenous stratagems to limit adverse aspects of altered iron handling may prove of therapeutic benefit for the treatment of SIRS and ALI.

systemic inflammatory response; hemolysis; oxidative stress

THE HOST RESPONSE TO INFECTION and other forms of tissue injury has been termed the systemic inflammatory response syndrome (SIRS). Although controversy exists concerning the optimal defining criteria for SIRS, traditionally these have reflected changes in thermoregulation (body temperature >38°C or <36°C), the emergence of cardiovascular (heart rate >90 beats/min) and respiratory (tachypnea >20 breaths/min or arterial Pco2 <4.3 kPa) instability, and alterations in white blood cell count (>12,000 cells/mm3, <4,000 cells/mm3, or the presence of >10% band-form neutrophils) (99). When SIRS is attributable to an identifiable infectious process, it is termed sepsis. Sepsis complicated by predefined organ system dysfunction, through tissue or systemic hypotension, is regarded as severe (4). Together, SIRS, sepsis, and septic shock have been termed the sepsis syndromes.

SIRS is seen in association with a wide variety of noninfective insults, including major trauma and surgical procedures, especially those necessitating cardiopulmonary bypass (CPB) (16). The incidence of SIRS is high, afflicting up to 33% of patients requiring hospital admission and as many as 70% after CPB. The majority of patients with SIRS and its sequelae who fail to survive succumb to multiple organ dysfunction syndrome (MODS), the commonest manifestation of which is acute lung injury (ALI) (123). Fewer patients with SIRS develop the extreme manifestation of ALI, termed the acute respiratory distress syndrome (ARDS). However, ARDS is relatively common in patients with sepsis and severe sepsis, in whom incidences of 40% and 80%, respectively, have been reported (14, 123). The management of patients with the sepsis syndromes remains largely supportive and is aimed at correcting cardiopulmonary abnormalities. Despite a rise in the provision of intensive care unit (ICU) facilities in the developed world, the sepsis syndromes together represent the leading cause of death in adult general ICUs, with an associated mortality of 30–45%, rising even higher in those who develop ALI/ARDS (49, 94).

Pathophysiology of Acute Lung Injury

ALI and ARDS complicate a wide variety of serious medical and surgical conditions, not all of which involve the lung directly. Both are defined by refractory hypoxemia associated with lung inflammation and increased pulmonary vascular permeability. The first widely accepted radiological and physiological criteria were developed by an American-European Consensus Conference (13), which recognized ALI and ARDS as separate, yet related, states on a continuum of pulmonary damage. To diagnose ALI and ARDS in a patient with one or more recognized risk factors three criteria should be fulfilled. First, new, bilateral, diffuse, patchy, or homogeneous pulmonary infiltrates consistent with pulmonary edema must be demonstrated on chest radiography. Second, there should be no clinical evidence that heart failure, fluid overload, or chronic lung disease is responsible for the infiltrates. Alternatively, if the pulmonary artery occlusion pressure is measured, then this should be <18 mmHg. Finally, an arterial P02-to-inspired O2 fraction ratio <300 mmHg defines ALI, and a ratio <200...
mmHg defines ARDS. Susceptibility to ALI/ARDS is determined in part by the nature of the precipitating condition. Thus some 40–60% of patients with sepsis, severe sepsis, and septic shock develop lung injury, regardless of the anatomic site of infection (49, 74, 124, 176). By contrast, only around 15% of trauma victims are so afflicted (49, 166). Estimates of the overall incidence of ARDS have varied from 1.5 to 75 cases per 100,000 population (103), although recent European epidemiologic data found the defining criteria to be fulfilled in 15.8% of patients requiring ICU admission because of acute respiratory failure of all causes (17). Some 16.1% required mechanical ventilation. Of those who developed ALI/ARDS, 65.4% of cases fulfilled the relevant criteria early in ICU admission and the remainder did so within a median of 3 days. Of those who developed ALI within the first 24 h, 54.4% evolved to ARDS. By contrast, only 18.4% of patients with established ARDS had preceding ALI (17). In North America, a high crude incidence of ALI (78.9 per 100,000 population) has been identified, the incidence increasing with age (16 per 100,000 for patients aged 15–19 yr, 306 per 100,000 for those aged 75–84 yr) (147). Overall, this study suggested that there are around 190,000 cases of ALI per year in the US, with 74,500 deaths and accounting for 3.6 million hospital days (147).

Oxidative Stress

Oxidative stress is an inevitable consequence of aerobic respiration and metabolism. The production of oxygen intermediates, or reactive oxygen species (ROS), with the potential to modulate an array of cell signaling processes and at higher concentrations to cause oxidative damage, characterizes oxidative stress. In normal, healthy individuals, such prooxidant effects are more than adequately balanced by an array of protective stratagems including endogenous and dietary antioxidants, together with removal and repair mechanisms. However, in the long term the effects of oxidative stress become more apparent, demonstrated in the aging process and also in disease states such as chronic hyperglycemia and cancer (65). Moreover, in circumstances where excessive oxidative stress occurs acutely, these protective stratagems can become rapidly overwhelmed, resulting in pronounced levels of oxidative damage to biomolecules. Oxidative stress also modifies physiological redox-based signaling responses with consequences for pro- and anti-inflammatory processes. However, such modifications are not limited to the actions of ROS alone; indeed, production of, and interactions with, allied reactive nitrogen (RNS) and halogen (RHS) species appear to be equally important in this regard (64).

Oxidative stress is thought to be central to the pathogenesis of ALI. Alveolar macrophages and recruited, activated neutrophils release ROS, which cause injury through interactions with proteins, lipids, and DNA. It is thought that excessive production of ROS in patients with ALI overwhelms endogenous antioxidant systems that normally regulate redox state within the lung (91). There is an established literature indicating that ARDS is associated with pronounced oxidative/nitrosative/halogenic modification of biomolecules of systemic and lung-specific origin (Table 1). Furthermore, numerous studies have demonstrated that antioxidant protective stratagems are deficient in this population (61, 112). In addition, genomic studies (see Genetic Predisposition) suggest that variation in aspects of potential host-mediated responses to reactive species and catalysts may represent predisposing factors for disease onset in patients with ARDS (89). Nevertheless, the extent to which the release of ROS/RNS/RHS contributes to the onset and progression of ALI remains a matter of debate, not least because such modifications per se are seen as a general consequence of tissue injury (65). However, a number of studies utilizing in vivo models have suggested a causative link (see Table 2).

Iron

Absorption, transport, and compartmentalization. A fundamental and yet frequently overlooked aspect of ROS/RNS/RHS biochemistry is the role of variable-valence transition metal ion catalysts in the generation and potentiation of oxidative stress. Iron is the most abundant such metal ion in the body and is frequently utilized within protein/enzyme complexes involved in the physiological metabolism, storage, and transport of oxygen. The ability of such transition metal ions to freely donate and accept electrons singly overcomes subatomic kinetic stabilization arising in molecular oxygen because of parallel electron spin. Iron thereby allows the energetically favorable activation of what is in fact a relatively unreactive free radical gas. When incorporated within protein systems iron-catalyzed reactions are regulated and purposeful, but if freed from such constraints low-molecular-mass iron catalyzes an array of inorganic and organic electron transfer reactions involving oxygen and/or nitrogen and halogen species that ultimately lead to the formation of aggressive and damaging reactive species capable of modifying biomolecules. Furthermore, iron is an absolute requirement for microbial growth, and invading microbes utilize diverse mechanisms to acquire iron from hosts. Finally, iron is a valuable resource for the body, being required for essential functions including biosynthesis and cellular proliferation. Despite this, iron is relatively poorly absorbed from the gut, and elaborate reprocessing is necessary to maintain and supplement specific iron pools. Balancing the body’s requirement for iron against potential adverse consequences therefore represents a physiological challenge, particularly in states of tissue injury or inflammation. Thus during episodes of sepsis the limitation of iron availability prevents deleterious ROS generation and counteracts microbial invasion and virulence.

A coordinated system facilitates this rapid sequestration and compartmentalization of liberated free iron both extra- and intracellularly (Fig. 1). Iron absorbed in the gut is immediately bound to transferrin, while that liberated by cellular lysis is tightly bound as ferric iron by circulating transferrin and lactoferrin. In addition, ceruloplasmin binds and converts ferrous iron to ferric iron for subsequent binding by transferrin. Furthermore, plasma haptoglobin and hemopexin bind iron-based hemoglobin and heme, respectively (59). Albumin, the most abundant plasma protein, has a nonspecific iron binding potential and one high- plus several low-affinity binding sites for heme, which it sequesters especially under conditions of haptoglobin depletion (7, 18). These specific binding molecules physically enclose the iron moieties and thus hinder prooxidant reactions (59, 62, 114). Moreover, macrophages, which are the primary cell type responsible for the removal of decompartmentalized iron, largely within the liver and reticu-
and reactive halogen species in patients with acute lung injury.

Table 1. Selection of research articles demonstrating presence of reactive oxygen species, reactive nitrogen species, and reactive halogen species in patients with acute lung injury

<table>
<thead>
<tr>
<th>Biological Source</th>
<th>Biomolecule Type</th>
<th>Modification</th>
<th>Reactive Species</th>
<th>Implications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic (plasma)</td>
<td>Plasma proteins; ceruloplasmin, transferrin, α(1)-protease inhibitor, α(1)-antichymotrypsin, β-chain fibrinogen</td>
<td>Tyrosine nitration</td>
<td>RNS</td>
<td>Impaired ceruloplasmin ferroxidase activity; impaired elastase inhibiting activity.</td>
<td>48</td>
</tr>
<tr>
<td>Lung (BAL fluid)</td>
<td>Protein</td>
<td>Carbonyl formation</td>
<td>ROS, RNS</td>
<td>Evidence of excessive oxidant formation in lungs</td>
<td>98</td>
</tr>
<tr>
<td>Lung (BAL fluid)</td>
<td>Protein</td>
<td>Ortho-tyrosine formation; tyrosine nitration</td>
<td>ROS</td>
<td>Evidence of neutrophil-derived oxidant formation</td>
<td>90</td>
</tr>
<tr>
<td>Lung (breath condensate)</td>
<td>Arachidonic acid</td>
<td>Tyrosine chlorination Isoprostane formation</td>
<td>RNS, RHS</td>
<td>Evidence of excessive oxidant formation in lungs</td>
<td>22</td>
</tr>
<tr>
<td>Systemic (plasma)</td>
<td>n-3 fatty acids</td>
<td>4-Hydroxy-2-nonenal formation</td>
<td>ROS</td>
<td>Evidence of excessive oxidant formation systemically</td>
<td>137</td>
</tr>
<tr>
<td>Breath condensate</td>
<td>ROS</td>
<td>Hydrogen peroxide</td>
<td>ROS</td>
<td>Evidence of excessive oxidant formation in lungs</td>
<td>159</td>
</tr>
<tr>
<td>Systemic (plasma)</td>
<td>Immunoglobulins</td>
<td>Nitrotyrosine</td>
<td>RNS</td>
<td>Antibodies to nitrated protein provide further evidence of nitrosative stress</td>
<td>165</td>
</tr>
<tr>
<td>Lung (BAL fluid)</td>
<td>Glutathione</td>
<td>Oxidized glutathione</td>
<td>ROS</td>
<td>Evidence of excessive oxidant formation in lungs</td>
<td>19</td>
</tr>
<tr>
<td>Lung (BAL fluid)</td>
<td>Nitrate/nitrite</td>
<td>RNS</td>
<td>Evidence of nitric oxide formation</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>Lung (BAL fluid)</td>
<td>Tyrosine nitration</td>
<td>RNS</td>
<td>Evidence of nitrosative stress; surfactant dysfunction</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>Systemic (plasma)</td>
<td>Protein</td>
<td>Thiol oxidation; carbonyl formation</td>
<td>ROS</td>
<td>Evidence of excessive oxidant formation systemically</td>
<td>135</td>
</tr>
</tbody>
</table>

ROS, reactive oxygen species; RNS, reactive nitrogen species; RHS, reactive halogen species; BAL, bronchoalveolar lavage.

loendothelial system, possess cell surface receptors specific for both haptoglobin and hemopexin and facilitate their removal (76, 129).

Once within cells, free iron is transported between compartments by a number of proteins. The most widely described are members of the natural resistance-associated macrophage protein (NRAMP) family, which are transmembrane divalent metal ion transporters (119). After uptake into the cytoplasm and mitochondria excess free iron is tightly bound within multimeric ferritin (168). Each ferritin subunit is capable of binding 4,500 iron molecules as insoluble Fe3+, which again prevents the metal ion from catalyzing deleterious redox reactions. The enzyme heme oxygenase (HO) is responsible for the metabolism of heme imported from the extracellular space, thereby liberating carbon monoxide, bilirubin, and ferrous iron. It is recognized that the antioxidant role of HO is in part dependent on the subsequent sequestration of iron within ferritin. Furthermore, excess HO activation may be deleterious in conditions of heme excess. In both compartments, iron levels are under tight control. The recently described peptide hormone hepcidin is released by the liver in response to iron excess (120). Hepcidin reduces the intestinal absorption of iron and promotes its retention by macrophages and hepatocytes via the inhibition of cell-membrane iron transport. Conversely, hepcidin production is suppressed by anemia and hypoxia, under which conditions increased erythropoiesis results in greater demand for iron. Within cells, free iron levels are tightly controlled by the iron regulatory proteins (IRPs) (20, 172). These RNA binding proteins affect the stability or translation of those mRNAs that encode proteins involved in cellular iron homeostasis, thereby controlling the uptake, utilization, and storage or export of iron. Dysregulation of all steps in this system is recognized to contribute to disease pathophysiology including SIRS and lung injury (44, 134).

Although the most abundant, iron is not the sole metal cation participating in cellular redox chemistry. Other transition metals such as copper and cobalt are also involved in superoxide generation and Fenton chemistry and are therefore also under tight regulation (119, 170). Furthermore, zinc, which is a fixed-valence transition metal, acts indirectly as an antioxidant. Its uptake is partly in competition with iron such that in iron-deficient states zinc absorption and its incorporation as zinc protoporphyrin are promoted in place of heme (iron protoporphyrin) (88). Although it is recognized that zinc protoporphyrin is an inhibitor of the heme catabolic enzyme HO, the physiological and pathophysiological significance of this interaction is not well described.

Iron and lung injury. Our own studies in patients with ALI (61, 111, 125, 136, 139) have shown that altered plasma and lung iron chemistry/mobilization are features of, and contribute
Evidence suggestive of prooxidant iron (60)- and hydroxyl radical-mediated obliteration and eventual graft failure (12, 15, 102, 131, 142). It appears to be closely linked to the development of bronchiolitis (43). Disruption of iron homeostasis in transplanted lung results in marked oxidative injury and pulmonary dysfunction. Evidence that instillation of iron or iron products into the lungs increases the potential for oxidant-mediated damage. XOR can contribute to lung inflammation by expression and conversion to the oxidant-producing enzymatic form in a mobile cell population.

Table 2. Selection of research articles detailing in vivo models demonstrating involvement of reactive species in development of acute lung injury

<table>
<thead>
<tr>
<th>Model</th>
<th>Challenge</th>
<th>Response</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated rabbit lungs</td>
<td>HOCl</td>
<td>Pulmonary hypertension and edema formation; Increased exhaled hydrogen peroxide and oxidized glutathione and lung leak</td>
<td>Evidence suggestive of oxidant formation linked to lung injury. Responses were decreased in the presence of antioxidants.</td>
<td>66–68</td>
</tr>
<tr>
<td>Rodent model</td>
<td>Tracheal instillation of IL-1</td>
<td>Neutrophil sequestration to the pulmonary vasculature, pulmonary edema, and tissue oxidation</td>
<td>IL-1-linked PAF production has implication for neutrophil influx, proinflammation, and oxidant product in this model of lung injury.</td>
<td>96</td>
</tr>
<tr>
<td>Mouse model</td>
<td>Immunotargeting of glucose oxidase to the vascular endothelium</td>
<td>Neutrophil sequestration to the pulmonary vasculature and pulmonary edema; increased myeloperoxidase activity, NF-κB activation, and hydrogen peroxide production</td>
<td>Localized production of hydrogen peroxide at the endothelium leads to manifestations of ALI and ARDS.</td>
<td>24</td>
</tr>
<tr>
<td>Rodent model</td>
<td>Tracheal instillation of PAF</td>
<td>Neutrophil sequestration to the pulmonary vasculature and pulmonary edema; increased myeloperoxidase activity, NF-κB activation, and hydrogen peroxide production</td>
<td>Loss of EC-SOD from alveolar parenchyma</td>
<td>122</td>
</tr>
<tr>
<td>Mouse model</td>
<td>Hyperoxia (72 h)</td>
<td>Loss of EC-SOD from alveolar parenchyma</td>
<td>Loss of a lung-specific antioxidant defense increases the potential for oxidant-mediated damage.</td>
<td>178</td>
</tr>
<tr>
<td>Rodent model</td>
<td>Intratracheal cytokine insufflation</td>
<td>Increased XOR levels in mononuclear phagocytes in lungs with evidence of increased oxidant formation</td>
<td>XOR can contribute to lung inflammation by expression and conversion to the oxidant-producing enzymatic form in a mobile cell population.</td>
<td></td>
</tr>
</tbody>
</table>

**ALL**: acute lung injury; **ARDS**: acute respiratory distress syndrome; **PAF**: platelet-activating factor; **EC-SOD**: extracellular superoxide dismutase; **XOR**: xanthine oxidoreductase.

to, the development and propagation of this acute inflammatory process. In studies by others, elevated serum levels of the iron storage protein ferritin in patients at risk of developing lung injury were found to be predictive for the subsequent development of ALI (27, 150). Serum ferritin is an indicator of body iron status. Studies utilizing bronchoalveolar lavage fluid have demonstrated increased levels of nonheme iron in patients with ALI compared with normal individuals (60) and elevated levels of iron and heme-binding proteins including ferritin, lactoferrin, transferrin, and haptoglobin (44). Furthermore, elevated expression of the heme-catabolizing enzymes HO-1 and -2 is detected in lungs of such patients (116), supporting a key role for iron turnover in pulmonary tissues. Moreover, a series of experiments in lung epithelium have identified iron uptake, detoxification, and excretion systems that may protect against iron-catalyzed oxidant production and the associated deleterious consequences (46).

However, despite such protection iron-mediated lung injury can occur, particularly in circumstances in which increased oxidant production is also present. Thus lung injury induced by ozone exposure has been shown to be an iron-dependent process (45), as have the effects of hyperoxia when HO-1 expression and activity are elevated (30, 157). There is also additional evidence that instillation of iron or iron products into the lungs results in marked oxidative injury and pulmonary dysfunction (43). Disruption of iron homeostasis in transplanted lung appears to be closely linked to the development of bronchiolitis obliterans and eventual graft failure (12, 15, 102, 131, 142). Evidence of prooxidant iron (60)- and hydroxyl radical-mediated protein modifications in the lungs suggests a role for a iron-catalyzed process in the pathogenesis of ALI (90).

**Heme/hemoglobin.** The presence of free hemoglobin within the circulation represents a considerable prooxidant risk. Oxidative reactions from the interaction of heme proteins (hemoglobin and myoglobin) with peroxides are known to occur, resulting in the formation of end products, including isoprostanes, that contribute to disease pathology (141). Furthermore, release of prooxidant iron from the heme moiety of hemoglobin occurs in the presence of peroxides (60). Hemolysis and hemoglobin release also lead to systemic nitric oxide (NO) deficiency (146), with adverse effects on vascular tone and coagulation. Thus hemoglobin rapidly reacts with free NO in an irreversible fashion, producing nitrate and methemoglobin. In addition, methemoglobin (but not heme or hemoglobin) activates endothelial cells to produce the proinflammatory chemokines interleukin (IL)-6 and -8, with membrane expression of E-selectin. These responses appear to be regulated via the expression of the cell signaling moiety NF-κB but are independent of direct effects of heme catabolism (101). Additionally, in vivo models have shown hemolysis to be associated with pronounced neutrophil activation and recruitment (55), the release of proinflammatory chemokines on exposure to the malarial pigment hemozoin in a model of malaria (75), and pronounced epithelial and endothelial cell destruction related to iron turnover and nitrosative/oxidative stress in a hemorrhagic model of lung trauma (54). Exposure of human neutrophils to the heme analog hemin significantly delays spontaneous neutrophil apoptosis (5, 110), thereby extending their...
proinflammatory potential. Finally, free heme and hemoglobin are microbial virulence factors (7, 37, 145).

The release of hemoglobin and heme as a result of hemolytic disease or injury therefore has the potential to mediate prooxidant, proinflammatory, and promicrobial responses. Infections including malaria, meningitis, and *Escherichia coli*, blood transfusion reactions, and surgery necessitating CPB are all associated with hemolytic events and are recognized risk factors for the development of ALI, possibly via these mechanisms (25, 31, 104, 121, 152, 154, 158). Moreover, chronic alcohol abuse is now recognized to be an independent risk factor for the development of ARDS (82, 115). Dysregulation of iron homeostasis is characteristic of chronic and acute liver disease/failure associated with alcoholism (162), as is elevated serum ferritin (also seen in established ARDS) (97). Aberrant iron processing and turnover by the liver in this population may contribute to the heightened risk for the development of ALI/ARDS.

**Iron-mediated cell signaling responses.** Changes in iron levels within cells can also modify signaling events during the inflammatory response. A direct role for iron as a cellular signaling agent is established; indeed, the activity of the IRPs is dictated by cellular iron levels. The IRPs regulate cellular iron uptake and storage at the posttranscriptional level via binding to iron-responsive elements encoded on several genes, including those for the transferrin receptor and ferritin. However, it is now apparent that other stimuli relevant to the onset of ALI, such as lipopolysaccharide and oxidative stress, can regulate IRP activity and hence cellular iron status (21, 169). Iron can signal independently of the IRPs, most likely via redox-based signaling processes. Indeed, the activity of the proinflammatory transcription factors NF-κB (80, 179), AP-1, and SP-1 (86, 167) have been shown to be influenced by either the supply of iron or its withdrawal. Moreover, the proinflammatory transcription factor hypoxia-inducible factor (HIF) is known to be regulated by iron as well as oxygen levels (113). Finally, iron signaling processes in endothelial cells have been described that dictate cellular fate. Iron uptake regulated via the transferrin receptor, coupled to increased levels of cellular oxidant production, mediates activation of caspase 3 and endothelial cell apoptosis (160). Such responses appear to be further regulated by NO (108).

It therefore appears that cellular iron can act as a proinflammatory signaling agent, with the potential to influence responses as diverse as chemokine production and cellular fate. Therefore, iron/heme/hemoglobin cellular uptake and subsequent metabolism may well contribute to the promotion of inflammation in certain patient groups at risk for ALI.

**Heme oxygenase.** The HO enzymes are key to the sequestration of iron in the sepsis syndromes and after hemolysis. HO-1, the inducible isoform of the enzyme, is upregulated by binding of hemoglobin/haptoglobin complexes to the specific CD163 macrophage receptor after CPB (129). In the nucleus heme binds to, and removes, a specific transcriptional repressor, BACH-1, from the HO-1 promoter region, thus inducing the enzyme (156). Moreover, prior induction of the enzyme has been shown to ameliorate the deleterious effects of heme in a number of models (79, 95, 117, 171). Numerous studies have

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Fig. 1. This scheme depicts extra- and intracellular iron/heme transport, uptake, and storage mechanisms together with the potential for altered cellular signaling responses. Hb, hemoglobin; HO, heme oxygenase; NRAMP, natural resistance-associated macrophage protein; IRP, iron regulatory protein.
shown that the induction of HO-1 is protective in ALI and MODS through antioxidant, anti-inflammatory, antiproliferative, and antiapoptotic mechanisms (148). Although these actions are largely ascribed to the generation of its products, carbon monoxide and bilirubin, there is also evidence that the metabolism of heme itself is a key anti-inflammatory function of the enzyme. Furthermore, it has recently been suggested that HO-1 is localized to the mitochondrion and may lead to the metabolism of the iron-containing cytochromes in the electron transport chain (28, 155). Thus the enzyme directly downregulates mitochondrial ROS production and enhances cellular tolerance to oxidative stress during inflammation. Finally, ferritin is coinduced with HO-1, suggesting that the sequestration of liberated iron is also essential to the enzyme’s protective function (149). In conditions of heme excess it has been suggested that the HO/ferritin system becomes overwhelmed and that the liberation of free iron may have deleterious consequences through the generation of prooxidant species (157).

Acute-phase response and iron sequestration. The physiological changes witnessed during the systemic inflammatory response arise in part as a result of cytokine induction following both infectious and noninfectious insults. Such physiological changes are also accompanied by the modulation of a wide range of plasma proteins, largely produced by the liver and collectively termed acute-phase response proteins (40). Included within this group are components of the complement, coagulation, and fibrinolytic systems, serum antiproteases and transport proteins, and others including granulocyte colony-stimulating factor, serum amyloid A, and, most widely used in the clinical setting, the C-reactive protein. Although these indexes of the acute-phase response may be useful for predicting outcome in patients with sepsis and ALI, their integrated role in the onset and resolution of such inflammation is not well understood.

The key modulators of iron homeostasis hepcidin, haptoglobin, hemopexin, lactoferrin, and ferritin, which promote iron sequestration, are all recognized as acute-phase proteins induced during inflammation (118, 151). Conversely, proteins that promote iron accumulation, transferrin and ferroportin, are suppressed. Together these changes have the effect of promoting a hypoferremic state (9). Thus during the acute-phase response the increase in iron-binding plasma proteins represents an active mechanism for the sequestration of excess iron rather than a purely passive defense. IL-1, IL-6, tumor necrosis factor-α, interferon-α, and interferon-γ are cytokines recognized to promote hypoferremia, thus denying microorganisms and neoplastic cells an essential growth factor and simultaneously minimizing the availability of iron for participation in deleterious prooxidant chemistry (11, 35, 47, 175). The coordinated induction of hypoferremia in response to a wide range of stimuli including bacterial and viral infection, malignancy, and extreme exercise suggests its fundamental importance as a protective mechanism during inflammation (72, 85, 163). Furthermore, the strength of the mechanism is highlighted during conditions of chronic inflammation, when it is now recognized that the prolonged elevation of hepcidin and its subsequent hypoferremic action are sufficient to induce the anemia of chronic disease (174).

Although the induction of hypoferremia represents an early response to infectious and noninfectious proinflammatory stimuli, there is evidence that this system may become overwhelmed and that a failure of iron sequestration and inappropriately elevated levels of tissue iron may contribute to disease pathogenesis.

Such decompartmentalized iron arises primarily from cell necrosis and lysis. The deleterious effects of iron release consequent upon hemolysis are recognized in both acute and chronic disease. Thus, after surgery, especially that involving CPB, and in severe fulminant infections such as malaria and E. coli, hemolysis may potentiate the inflammatory response (63, 87, 154). Additionally, in chronic hemolytic conditions such as sickle-cell disease and the thalassemias, iron release and sequestration contribute to disease pathogenesis (10, 173). By contrast, the release of myoglobin during rhabdomyolysis and subsequent acute renal tubular necrosis is perhaps the most widely described example of nonhematologic iron decompartmentalization contributing to tissue injury (3, 36). Free iron is also implicated in the pathogenesis of a wide variety of other tissue injuries following necrosis (83, 130, 143, 185). Finally, there is some evidence that under certain circumstances iron is transported from cells by specific mechanisms during inflammation, although the mechanisms for this are not fully understood (46, 73).

Cardiopulmonary bypass, hemolysis, SIRS, and development of ALI. SIRS is a common complication of surgery necessitating CPB, but the reasons for its development are not well understood. Iron mobilization appears to be associated with SIRS. ALI is associated with CPB (26), during which hemolysis occurs with release of heme, the primary source of such iron. Moreover, acute hemolysis is a recognized feature of several infectious diseases associated with severe sepsis and ARDS. Despite this, the contribution of hemolysis to morbidity has yet to be explored, and it is considered a marker of illness severity rather than a target for therapeutic intervention. However, removal of iron would theoretically both deny infecting organisms an essential probiotic and reduce prooxidant stress. Strategies that have sought to reduce hemolysis during CPB include changes in pump design and surgical practice, which have been associated with reduced indexes of inflammation and morbidity (93, 154, 177).

Although hemolysis is a common feature of CPB and likely to contribute to the manifestation of SIRS, only a small minority of patients go on to develop ALI and/or severe sepsis. It therefore seems likely that a predisposition exists in a minority of at-risk individuals for the development of these life-threatening syndromes.

Genetic Predisposition

Prospective studies have indicated that only a proportion of individuals at risk of ARDS go on to develop the syndrome, ranging from 14% to 40% in patients suffering trauma (166) and sepsis (124), respectively, thereby raising the possibility of genetic susceptibility. This is a complex hypothesis to explore, not only because of the incomplete penetrance of genetic traits and heterogeneity of the loci involved but also because of the influences of variable, nongenetic risk factors and of treatment strategies. Any genetic association must therefore be stratified by potential gene-gene and gene-environment interactions. Recent advances in molecular genetics and bioinformatics have enhanced the study of complex genetic disease. Most sequence variation in DNA is present at the single base level [e.g.,
an allele frequency of microsatellites. SNPs are defined as variations in DNA fixed at mapping required and their relative stability compared with choice for candidate gene studies because of the high density nucleotide polymorphism (SNP) analysis is the method of because of the sporadic nature of the syndrome. Candidate familial linkage studies are not feasible in patients with ALI produced with the release of the first-generation copy number magnitude. A further level of complexity has recently been intro-
duced as DIPs and repeat regions of variable magni-
tude. These polymorphisms have a role to play in patients with sepsis, a risk factor to increased risk of bronchial asthma (78) and to mortality from tuberculosis (84). NADPH oxidase and catalase polymor-
phisms have a role to play in patients with sepsis, a risk factor for ALI (126).

Individual variation in the way extracellular iron, heme, and hemoglobin are handled in response to specific insults, together

Table 3. Genetic polymorphism studies in ALI/ARDS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Association</th>
<th>Author (year)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>DIP</td>
<td>ACE deletion associated with mortality in ARDS</td>
<td>Adamzik (2007)</td>
<td>1</td>
</tr>
<tr>
<td>ACE</td>
<td>DIP</td>
<td>Deletion homozygotes associated with susceptibility to and outcome of ARDS</td>
<td>Marshall (2002)</td>
<td>105</td>
</tr>
<tr>
<td>CC16</td>
<td>−26 G/A</td>
<td>Negative</td>
<td>Frerking (2005)</td>
<td>38</td>
</tr>
<tr>
<td>FTL</td>
<td>Multiple SNPs</td>
<td>−3381 GG associated with ARDS of extrapulmonary origin</td>
<td>Lagan (2008)</td>
<td>89</td>
</tr>
<tr>
<td>HMOX2</td>
<td>Multiple SNPs</td>
<td>Common haplotype decreased in ARDS of pulmonary origin</td>
<td>Lagan (2008)</td>
<td>89</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>241 Gly/Arg</td>
<td>C allele and CC genotype associated with mortality in both ARDS and ICU patients</td>
<td>Quasney (2002)</td>
<td>133</td>
</tr>
<tr>
<td>IL-6</td>
<td>−174 G/C</td>
<td>251A trend to increased incidence of posttraumatic ARDS</td>
<td>Hildebrand (2007)</td>
<td>71</td>
</tr>
<tr>
<td>IL-8</td>
<td>−251 A/T</td>
<td>−1082 G/A</td>
<td>Gong (2006)</td>
<td>50</td>
</tr>
<tr>
<td>MBL-2</td>
<td>Multiple SNPs</td>
<td>Codon 54 BB genotype associated with ARDS risk and outcome</td>
<td>Gong (2007)</td>
<td>53</td>
</tr>
<tr>
<td>MIF</td>
<td>Multiple SNPs</td>
<td>3’ haplotypes associated with sepsis and sepsis-ALI</td>
<td>Gao (2007)</td>
<td>41</td>
</tr>
<tr>
<td>MLCK</td>
<td>Multiple SNPs</td>
<td>Four SNPs associated with sepsis in European Americans and one with ALI</td>
<td>Gao (2006)</td>
<td>42</td>
</tr>
<tr>
<td>NFKB1</td>
<td>−94 ATTG DIP</td>
<td>Deletion allele associated with more severe ARDS</td>
<td>Adamzik (2007)</td>
<td>2</td>
</tr>
<tr>
<td>NFKBIA</td>
<td>−881 A/G, −826 C/T, and −297 C/T</td>
<td>GTC haplotype confers susceptibility to ARDS</td>
<td>Zhai (2007)</td>
<td>184</td>
</tr>
<tr>
<td>NRF2</td>
<td>Multiple SNPs</td>
<td>−617A associated with increased risk of ALI after trauma</td>
<td>Marzec (2007)</td>
<td>107</td>
</tr>
<tr>
<td>PBEF</td>
<td>−1001 T/G, −1543 C/T</td>
<td>−1001G allele and −1543C haplotype associated with increased risk of ARDS and ICU mortality</td>
<td>Bajwa (2007)</td>
<td>8</td>
</tr>
<tr>
<td>PBEF</td>
<td>−1001 T/G, −1543 C/T</td>
<td>Associated with increased risk of ALI</td>
<td>Ye (2005)</td>
<td>181</td>
</tr>
<tr>
<td>SP-B</td>
<td>Intron 4 repeats</td>
<td>Associated with increased risk of ARDS in females</td>
<td>Gong (2004)</td>
<td>51</td>
</tr>
<tr>
<td>SP-B</td>
<td>+1580 T/C</td>
<td>+1580C allele associated with ARDS</td>
<td>Lin (2000)</td>
<td>100</td>
</tr>
<tr>
<td>TNF</td>
<td>−308 G/A</td>
<td>−308A associated with increased 60-day mortality in ARDS</td>
<td>Gong (2005)</td>
<td>52</td>
</tr>
<tr>
<td>VEGF</td>
<td>−460 C/T, +405 C/G, and +936 C/T</td>
<td>+936TT and haplotype TCT associated with increased mortality in ARDS</td>
<td>Zhai (2007)</td>
<td>183</td>
</tr>
<tr>
<td>VEGF</td>
<td>+936 C/T</td>
<td>+936 T allele carriage associated with susceptibility to ARDS</td>
<td>Medford (2005)</td>
<td>109</td>
</tr>
</tbody>
</table>

DIP, deletion/insertion polymorphism; SNP, single nucleotide polymorphism; ICU, intensive care unit.
with cellular uptake and processing mechanisms, may also represent candidates for predisposition studies in this patient population. Indeed, a complex iron regulatory network is mobilized in response to extracellular iron, heme/hemoglobin release, which, if responsive in an abnormal way, could contribute to aberrant, iron/heme-mediated inflammation (Fig. 1). Furthermore, studies have indicated that variation in basal iron status is evident in wild-type strains of mice. Analysis of murine quantitative trait loci has identified several polymorphic genes involved in iron handling close genetic relatives of which can be found in humans (56). Genes encoding iron-metabolizing proteins have been shown to be polymorphic through bioinformatics and sequencing (32). There are known mutations and polymorphisms that affect the functionality of genes encoding iron-metabolizing proteins (34, 92).

In preliminary studies, we found evidence of SNPs in a panel of nine genes encoding iron-metabolizing proteins in a case control study of 123 patients with ARDS. A role for polymorphisms of the Ferritin Light chain gene in susceptibility to ARDS of extrapulmonary origin and for HO-2 gene in a protective role against ARDS of pulmonary origin emerged (89). While the functional significance of these data is at present not clear, they strongly indicate that variation in the iron-regulatory response at the genotypic level may be a significant predisposing factor in the development of ARDS, the most severe manifestation of the sepsis syndromes.

Large-scale prospective cohort studies are already planned on ICU “at-risk” surgical groups (51, 107). Careful characterization of phenotypes in these cohorts will avoid dilution of the underlying genotype effect. An approach to candidate gene studies is also evolving that uses hypothesis-generating tools such as expression arrays and time series analysis of involved tissues and cells, which should help to direct candidate gene research together with utilization of tools such as HapMap (77a) and CNPmap (140).

Iron Decompartmentalization and Clinical Presentation

Acute-phase changes in iron homeostasis that characterize proinflammatory responses act to ameliorate the effects of inflammation. A reduction in the potential for microbial growth and for uncontrolled iron-catalyzed oxidant production would seem to be the ultimate goal of the observed hypoferricmic state. However, as illustrated in the previous sections of this review, the presentation of acute inflammatory conditions including SIRS and life-threatening ALI is accompanied by perturbations in body iron and heme status that contribute to both microbial growth and excessive oxidative stress. The compromise of systemic mechanisms that usually act to specifically limit iron availability enhances the potential for damaging ROS production, and this has implications for endothelial-vascular damage and dysfunction and accompanying organ failures. Decompartmentalization of iron/heme will further impact on compromised defenses, with potential adverse implications. Oxidative damage directly alters the function of modified biomolecules, and, moreover, some end products formed (e.g., aldehydes from lipid oxidation) may be cytotoxic at high concentrations and bioactive at lower concentrations (33). Thus lung-orientated lipid peroxidation is likely to generate chemotactic end products, promote lung neutrophilia, and enhance proinflammatory signaling between other inflammatory cells. Even cellular iron uptake in the lung is fraught with risk. In particular, transferrin receptor-mediated uptake of transferrin-bound iron by epithelial cells increases the labile reactive iron pool within the cytosol and not the safe storage of iron in ferritin, thereby rendering these cells extremely vulnerable to oxidative damage and or altered redox-based signaling responses that may be either pro- or anti-inflammatory (46). There are also implications for cellular fate and remodeling, because iron is known to modulate apoptosis and promote fibro-proliferation in both liver and lung (39, 128, 182). Therefore, aspects of pulmonary hypertension and the organizational phase of ARDS may be influenced by such mechanisms (146, 164). The roles of heme metabolism and HO-1 induction in neutrophils and antiapoptotic responses are also important in this regard.

It seems apparent that release, uptake, metabolism, and storage of iron/heme during inflammation may well have profound implications for disease onset and progression in those who are in some way predisposed to develop these syndromes. Figure 2 outlines the means by which iron/heme decompartmentalization is modulated and impacts on inflammation in at-risk populations for the onset and progression of SIRS, ALI, and indeed other organ injury.

Pharmaceutical Interventions

Many investigations have been performed to evaluate the potential of pharmacotherapeutic interventions for the treatment of ALI (23). Most were designed to reduce cellular and mediator-driven inflammation. In the light of some of the data presented above, attempts have been made to introduce antioxidative agents to improve mortality, in particular employing the scavenging antioxidant and glutathione precursor N-acetylcysteine (NAC), but have failed to demonstrate a survival benefit or even a reproducible effect on pulmonary physiology. Human serum albumin (HSA), a pharmaceutical in common use in the critical care setting, also has antioxidant functions and has been shown to improve plasma antioxidant status in patients with ALI (138). Interventions specifically targeting the prooxidant and promicrobial properties of low-molecular-mass iron and free heme/hemoglobin have not yet been investigated in patients with ALI. However, in vivo studies have been performed (144) in which the combined use of NAC and the iron chelator deferoxamine were shown to ameliorate in an additive fashion proinflammatory indexes in rodent models of sepsis and ARDS. While these results seem logical and encouraging, translation to human-based studies may well not be appropriate. Thus NAC in high doses can aggravate prooxidant responses via redox cycling of transition metal ions. Furthermore, deferoxamine is a particularly potent iron chelator, and its use in individuals who are not iron overloaded may deplete essential iron stores in excess of those iron pools that are contributing to unwanted prooxidant responses. However, the advent of new, more specific iron chelators may now improve the feasibility of such a therapeutic approach. Perhaps a better way to limit the effects of extracellular iron mobilization would be to utilize or augment interventions based on in vivo binding and removal mechanisms. Potential therapies could include the iron-binding proteins transferrin and lactoferrin for the removal of free redox active iron. As for limiting iron-mediated effects related to hemolysis, heme removal could be achieved with hemopexin, a heme-binding protein.
with antioxidant function (62), and an associated in vivo removal system or with HSA, which is also an efficient heme-binding protein (127) that has established therapeutic usage in the critically ill, albeit for different reasons than those suggested here. Finally, free hemoglobin is avidly bound by haptoglobin and removed from the circulation mainly via the CD163 receptor of macrophages. Haptoglobin also has reported antioxidant functions (58), and transgenic mice that overexpress haptoglobin have demonstrated a markedly protective response against hemoglobin-induced lung injury (180). Indeed, replacement of haptoglobin has been studied as a protective stratagem during CPB, with limited results (69, 161). Trials in patients at greater risk of developing ALI may prove more fruitful and provide a paradigm for the treatment of those diseases associated with severe sepsis and organ dysfunction in which hemolysis is present.

While the reasoning proposed for the use of antioxidant therapy to treat patients with ARDS seems to be justifiable, caution is required. The potential pitfalls associated with chelation therapy have been mentioned, but limiting the effects of oxidative stress directly may also alter both pro- and anti-inflammatory redox signaling responses (29). The use of antioxidants may therefore limit not only unwanted ROS-mediated responses but also the induction of protective endogenous stratagems. A targeted approach with defined aims therefore seems appropriate. In this sense, the predisposing insult will determine the nature of the therapeutic approach adopted, and the timing and duration of any intervention would be of crucial importance. Prophylactic use in “at-risk” groups may prove a more fruitful approach than administration to those with established disease. Perhaps the lack of success reported, in terms of survival benefit, with previous antioxidant trials is related to such shortfalls.

**Summary**

Iron mobilization and decompartmentalization may be significant in the pathogenesis of SIRS and associated organ system failure, specifically ALI. Experimental and clinical investigations suggest that iron-catalyzed oxidative stress and the proinflammatory effects of hemolysis, coupled to decreased antioxidant, anti-inflammatory moieties, lead to increased cytotoxicity in patients with SIRS and ALI. Moreover, such redox imbalance has implications for the modulation of signaling pathways relevant to the inflammatory response, especially in genetically predisposed populations. Specific thera-

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Fig. 2. This scheme summarizes the mechanisms by which iron decompartmentalization contributes to the propagation of the inflammatory response and organ dysfunction. The hypoferric response (see Fig. 1) attenuates these effects and may be influenced by genetic variability in at-risk populations. CPB, cardiopulmonary bypass.
peutic interventions based on endogenous stratagems may emerge from these hypotheses.

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