ANTIOXIDANTS & REDOX SIGNALING
Volume 34, Number 12, 2021

DOI: 10.1089/ars.2019.7964

Discoveries

FORUM REVIEW ARTICLE

Dual Role of Hydrogen Peroxide as an Oxidant in Pneumococcal Pneumonia

Mobarak Abu Mraheil,[1] Haroldo A. Toque,[2,3] Luigi La Pietra,[1] Juerg Hamacher,[4–6] Tenzing Phanthok,[7] Alexander Verin,[2,7] Joyce Gonzales,[7] Yunchao Su,[3] David Fulton,[2,3] Douglas C. Eaton,[8] Trinad Chakraborty,[1] and Rudolf Lucas[2,3,7]

Abstract

Significance: *Streptococcus pneumoniae* (*Spn*), a facultative anaerobic Gram-positive human pathogen with increasing rates of penicillin and macrolide resistance, is a major cause of lower respiratory tract infections worldwide. Pneumococci are a primary agent of severe pneumonia in children younger than 5 years and of community-acquired pneumonia in adults. A major defense mechanism toward *Spn* is the generation of reactive oxygen species, including hydrogen peroxide (H_2O_2), during the oxidative burst of neutrophils and macrophages. Paradoxically, *Spn* produces high endogenous levels of H_2O_2 as a strategy to promote colonization.
Recent Advances: Pneumococci, which express neither catalase nor common regulators of peroxide stress resistance, have developed unique mechanisms to protect themselves from H_2O_2. *Spn* generates high levels of H_2O_2 as a strategy to promote colonization. Production of H_2O_2 moreover constitutes an important virulence phenotype and its cellular activities overlap and complement those of other virulence factors, such as pneumolysin, in modulating host immune responses and promoting organ injury.
Critical Issues: This review examines the dual role of H_2O_2 in pneumococcal pneumonia, from the viewpoint of both the pathogen (defense mechanisms, lytic activity toward competing pathogens, and virulence) and the resulting host–response (inflammasome activation, endoplasmic reticulum stress, and damage to the alveolar–capillary barrier in the lungs).
Future Directions: An understanding of the complexity of H_2O_2-mediated host–pathogen interactions is necessary to develop novel strategies that target these processes to enhance lung function during severe pneumonia. *Antioxid. Redox Signal.* 34, 962–978.

Keywords: hydrogen peroxide, pneumococci, pneumonia, pyruvate oxidase, virulence factor, ARDS

Introduction

INFECTIONS OF THE LOWER RESPIRATORY TRACT represent the main cause of infectious disease mortality and represent the fifth highest cause of death overall. In 2015, pneumonia accounted for 2.7 million deaths worldwide (47). *Streptococcus pneumoniae* (*Spn*) is a major etiologic agent of community-acquired pneumonia and the leading cause of death in children younger than 5 years, worldwide (50, 51). It is a facultative anaerobe gram-positive bacterium that can

[1]Institute for Medical Microbiology, Justus-Liebig University, Giessen, Germany.
[2]Vascular Biology Center and [3]Department of Pharmacology and Toxicology, Medical College of Georgia at Augusta University, Augusta, Georgia, USA.
[4]Internal Medicine and Pneumology, Lindenhofspital, Bern, Switzerland.
[5]Lungen- und Atmungsstiftung Bern, Bern, Switzerland.
[6]Internal Medicine V—Pneumology, Allergology, Respiratory and Environmental Medicine, Faculty of Medicine, Saarland University, Saarbrücken, Germany.
[7]Department of Medicine, Medical College of Georgia at Augusta University, Augusta, Georgia, USA.
[8]Department of Medicine, Emory School of Medicine, Atlanta, Georgia, USA.

colonize the upper respiratory tract as a commensal bacterium in healthy individuals. This asymptomatic transitory phase can lead to severe pneumonia upon migration of bacteria to the lower respiratory tract (119). Infections with *Spn* can lead to meningitis after crossing the blood–brain barrier and the following entry into the blood stream can contribute to heart disease by causing microlesions in the myocardium (14).

Currently, two main types of vaccines have been developed to reduce or eliminate the burden of infections, that is, the unconjugated 23-valent polysaccharide vaccine and a 10- or 13-valent-conjugated polysaccharide vaccine. However, their serotype coverage is not universal and they are limited with respect to noncapsulated *Spn* (12, 97). Once symptoms of pneumonia are diagnosed, antibiotic therapy with amoxicillin for ages under five, and azithromycin or clarithromycin for ages over five is indicated (38, 71). Antibiotic therapy, however, needs to be initiated before breakdown of the alveolar–capillary barrier and systemic inflammation in pneumococcal pneumonia (8). Unfortunately, in view of emerging pathogens, demographic shifts toward older populations, and increasing antibiotic resistance, the occurrence of pneumonia is poised to worsen rather than improve in the coming years (30, 119).

Significance

Bacterial pneumonia represents one of the major comorbidities that occur with the acute respiratory distress syndrome (ARDS) (23, 48, 81). One of the major lethal complications of ARDS is the development of pulmonary edema. Indeed, pathological specimens from ARDS patients reveal diffuse alveolar damage, and animal studies of bacterial pneumonia-associated ARDS have demonstrated both alveolar epithelial and lung endothelial injury with accumulation of protein-rich fluid in the alveolar space (27, 55, 78, 88). The ability of *Spn* to promote lung disease in the human host depends not only on microbial virulence factors, such as the pore-forming toxin pneumolysin (Ply) (77, 79, 111, 137), but also on variables in the host (age, genetic, and environmental factors). These affect the capacity of the immune system to clear bacteria and the susceptibility to develop tissue damage (30, 61).

While there has been focus on the pathogens causing pneumonia in recent years, for example, on *Spn*, there is a high and urgent need for research from the perspective of the host. The absence of alveolar neutrophilia was shown to be deleterious in murine pneumonia models (8, 101) and has a high negative predictive value for bacterial pneumonia in critically ill patients with suspected infection (129). Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), play an important but highly complex role in pneumococcal pneumonia-associated ARDS. On the one hand, they are involved in neutrophil- and alveolar macrophage-mediated antibacterial defense, but on the other hand, they can contribute to the dysfunction of the alveolar–endothelial barrier and impair alveolar liquid clearance mechanisms. The following chapters discuss the actions of H_2O_2 from the viewpoint of the pathogen as well as in the host and outline its roles in driving the unfolded protein response (UPR), in blunting immune defense mechanisms, and in promoting acute lung injury.

Recent Advances: Actions of H_2O_2 on *Spn*

Spn *generates and exploits* H_2O_2

Spn, which grows predominantly in an aerobic environment of the oxygen-rich airway surface, can endogenously generate millimolar levels of H_2O_2 as a by-product during aerobic growth (90, 104). Here we describe those enzymes involved in pneumococcal H_2O_2 generation and examine how the pathogen exploits these high H_2O_2 levels to promote its colonization and enhance its virulence.

Enzymes involved in H_2O_2 generation in *Spn*. Pyruvate oxidase (SpxB) is considered the main source of H_2O_2 in *Spn*, as strains lacking this enzyme produce 87% less H_2O_2 (74). SpxB catalyzes the conversion of pyruvate to the phosphoryl donor, acetyl phosphate, and releases CO_2 and H_2O_2 as by-products (104). Lactate oxidase (LctO), which converts lactate to pyruvate, positively impacts pyruvate flux through SpxB. *Spn* mutants lacking lactate oxidase also produce significantly lower levels of H_2O_2 (38% of the total H_2O_2) (74, 90, 104, 122) (Overview in Table 1).

Deletion of *spxB* and *lctO* significantly reduces bacterial H_2O_2 production. The absence of these enzymes renders *Spn* highly susceptible to the presence of exogenous H_2O_2 stress generated by host phagocytic cells to facilitate clearance of bacteria at infection sites (74, 104). This suggests a link between endogenous bacterial H_2O_2 generation and resistance to exogenous H_2O_2 produced by cells of the immune system. Levels of adenosine triphosphate (ATP) decreased more rapidly in Δ*spxB* pneumococcal mutants than in the wild-type strain during H_2O_2 exposure, suggesting that the increased killing of these mutants is due to their inability to limit depletion of ATP during H_2O_2 stress (104).

High levels of secreted H_2O_2 promote *Spn* colonization. Endogenously generated H_2O_2 diffuses rapidly through cell membranes and accumulates in the extracellular milieu of *Spn* cultures in concentrations high enough to kill or inhibit other common inhabitants of the respiratory tract, such as *Haemophilus influenzae*, *Staphylococcus aureus*, *Neisseria meningitidis*, and *Moraxella catarrhalis* (103). Using an *in vitro* model mimicking *Spn*/*S. aureus* contact during colonization of the nasopharynx, pneumococcal SpxB/LctO-produced H_2O_2 was shown to be converted into the more potent oxidant hydroxyl radical ($^{\bullet}OH$) that rapidly kills *S. aureus* (139). Thus, high levels of H_2O_2 provide a competitive advantage to *Spn* over other pathogens and commensal bacteria. A similar strategy is also applied by lactic acid bacteria at other epithelial interfaces, such as in the gut or the vagina. Thus, excretion of high levels of H_2O_2 by vaginal lactobacilli (*e.g.*, *L. gasseri*, *L. acidophilus*, and *L. johnsonii*) represents one of the most important defense mechanisms against vaginal colonization by undesirable microorganisms (58, 85).

Adaptation to endogenously generated H_2O_2 in *Spn*. Lisher *et al.* (74) revealed that SpxB/LctO-generated H_2O_2 functions as an intracellular signaling molecule in *Spn* that modulates glycolytic-, nucleotide-, and capsule-biosynthesis *via* protein sulfenylation. The authors demonstrated a clear correlation between sulfenylation levels and endogenous H_2O_2 production, with SpxB and LctO being among the identified targets of protein sulfenylation. Sulfenylation of

Table 1. Overview of Pneumococcal Enzymes Involved in the Generation of and Protection from Hydrogen Peroxide

H_2O_2 producing enzymes		
	Mode of action	*Regulated by*
SpxB	Pyruvate + Pi + O_2 → H_2O_2 + CO_2 + acetyl-P	SpxR
LctO	Lactate + O_2 → H_2O_2 + pyruvate	ND
H_2O_2 detoxification		
	Impact on H_2O_2	*Mode of action*
TpxD	Direct	Elimination of H_2O_2 by reduction	CodY (activator)
NOX	Indirect	Conversion of O_2 to H_2O and thereby prevention of the generation of superoxide anion ($O_2^{\bullet-}$) and H_2O_2	ND
SodA	Indirect	Protection against H_2O_2 by removing $O_2^{\bullet-}$ and prevention of redox cycling of iron	ND
Dpr	Indirect	Inhibition of Fenton reaction by chelating free iron	ND[a]
Repair mechanisms against H_2O_2		
	Consequences of deletion on H_2O_2 sensitivity	
HtrA	Increased	CiaRH
ClpP	Increased	NmlR/MerR
CTM proteins	Increased (deletion of the operon *ccdA-tlpA-mrsAB*)	ND

[a]PerR-regulated in other bacteria (*Spn* lacks PerR).

ATP, adenosine triphosphate; CiaRH, two-component system composed of CiaH histidine kinase and CiaR response regulator; ClpP, caseinolytic ATP-dependent proteases; CodY, GTP-sensing transcriptional pleiotropic repressor; CTM proteins CcdA, putative cytochrome *c*-type biogenesis protein homologue; Dpr, Dps-like peroxide resistance; H_2O_2, hydrogen peroxide; HtrA, high-temperature requirement A; LctO, lactate oxidase; MerR, mercury-sensing regulatory protein; MsrAB, methionine sulfoxide reductase A/B protein; ND, not determined; NmlR, Neisseria merR-like regulator; NOX, NADH oxidase; SodA, superoxide dismutase; *Spn*, *Streptococcus pneumonia*; SpxB, pyruvate oxidase; spxR, *Streptococcus* pyruvate oxidase regulator; TlpA, thioredoxin-like protein family; TpxD, thiol peroxidase.

SpxB was proposed to allow it to function as an H_2O_2 ''sink,'' which is consistent with the fact that Δ*spxB* strains are more sensitive to exogenous H_2O_2 (74). Similarly, the thiol peroxidase (TpxD), which limits sulfenylation, was also shown to play a critical role in the adaptation to endogenous H_2O_2 *in Spn* (74).

A serine/threonine kinase StkP phosphorylates the response regulator ComE to control different cellular processes, including H_2O_2 production (107). Deletion of *comE* and *stkP* reduced the expression of *spxB* and *tpxD* and significantly decreased H_2O_2 generation (107).

A role for pneumococcal flavin reductase in resistance to oxidative stress has also been suggested, since an inactive mutant of the enzyme significantly increased susceptibility to H_2O_2, compared with wild-type *Spn*. Flavin reductase activity in *Spn* might also to be involved in bacterial virulence, as its absence does not affect *Spn* phagocytosis by primary mouse peritoneal macrophages, but it blunts adhesion to the type II alveolar-like cell line A549 (94).

Endogenous H_2O_2 promotes release of the pore-forming toxin Ply. The 53-kDa cholesterol-dependent pore-forming cytolysin (cholesterol-dependent cytolysin [CDC]) Ply (77, 79, 111, 137) can be released in the lungs by autolysis or upon antibiotic-mediated lysis (2). Although Ply lacks the N-terminal signal sequence found in other CDCs, which allows for extracellular release *via* the Sec-dependent pathway (128), the toxin's release has also been suggested in the absence of autolysis (4). Interestingly, *Spn* mutants that lack the pyruvate oxidase gene (*spxB*) are defective in Ply release. However, whereas complementation of *spxB* restored Ply release, exogenous addition of H_2O_2 failed to induce it. Since catalase supplementation prevented Ply release in some strains, these results indicate that intracellularly generated rather than secreted H_2O_2 promotes Ply release in some *Spn* strains (17).

Another link between SpxB and Ply release has been recently described. Deoxycholate-induced autolysis of *Spn* was significantly reduced in an SpxB-deficient strain, indicating a possible weakening of the cell membrane when SpxB is expressed (17). Endogenous H_2O_2 was found to control membrane fatty acid composition, by specific oxidation of the active site cysteine thiol residue of FabF (7). Nevertheless, it remains to be shown as to whether H_2O_2 generated by SpxB

affects the Ply release directly or rather indirectly, by affecting the expression of yet unknown pneumococcal activators or repressors.

Protective pneumococcal mechanisms against H_2O_2

Generation of ROS is a conserved strategy of host phagocytic cells, primarily neutrophils, monocytes, and macrophages, to facilitate clearance of bacteria at the infection site. Bacteria can be engulfed and enclosed in phagosomes, into which superoxide is released by activated NADPH oxidase 2 (Nox-2). The resulting superoxide $O_2^{-\bullet}$ can then be dismutated to H_2O_2 by superoxide dismutase (SOD) in macrophages, and H_2O_2 can further be converted by myeloperoxidase (MPO) in neutrophils to generate the highly microbicidal species, hypochlorous acid (64, 136). Combating endogenous and host innate immune cell-mediated oxidative stress is an integral aspect of colonization and virulence of the facultative anaerobe *Spn*.

Pneumococcal enzymes involved in defense from high H_2O_2 levels. Common proteins known to protect against oxidative stress in other bacterial species, such as the H_2O_2 scavengers catalase and NADPH-peroxidase, are absent in *Spn* (62, 123). However, previous studies have identified other pneumococcal enzymes implicated in the defense against oxidative stress (Table 1; Fig. 1), such as TpxD (53), Nox (3), SOD (143), and alkyl hydroperoxidase (alkyl hydroperoxide reductase [AhpD]) (100). The pneumococcal TpxD plays an important role in H_2O_2 elimination by catalyzing its reduction (53, 54).

Exposure to exogenous H_2O_2 (10–1000 μM) significantly upregulated the expression of *tpxD* in *Spn*, whereas deletion of *tpxD* decreased the growth rate and survival of pneumococci in the presence of exogenously added H_2O_2. Addition of the H_2O_2 scavenger catalase in the culture medium reversed the attenuated growth of the *tpxD* deletion mutant (53, 54). However, *Spn* lacks the transcription factors OxyR and PerR found in other bacteria such as *Escherichia coli*, which are described to regulate the expression of *tpxD* in response to H_2O_2 (18, 21, 62, 92). Instead, the transcription factor CodY was recently identified to be an activator of *tpxD* expression, triggering its upregulation in *Spn* under H_2O_2 stress (54).

The pneumococcal Nox is suggested to be indirectly involved in H_2O_2 scavenging, as it converts O_2 to H_2O and thereby prevents the generation of superoxide anion ($O_2^{\bullet-}$) and H_2O_2, which occurs when O_2 is not fully reduced. Pneumococcal Nox is necessary for *Spn* virulence in the murine respiratory tract and in Mongolian gerbil otitis media infection models (146). Unexpectedly, Nox mutations are linked to changes in virulence after intraperitoneal infection, where oxygen tension is low (3). However, Nox has also been associated with other functions such as *Spn* adhesion to A549 epithelial cells (96).

SODs protect cells from the toxic effects of $O_2^{\bullet-}$ by converting it into the more diffusible and less reactive H_2O_2 and

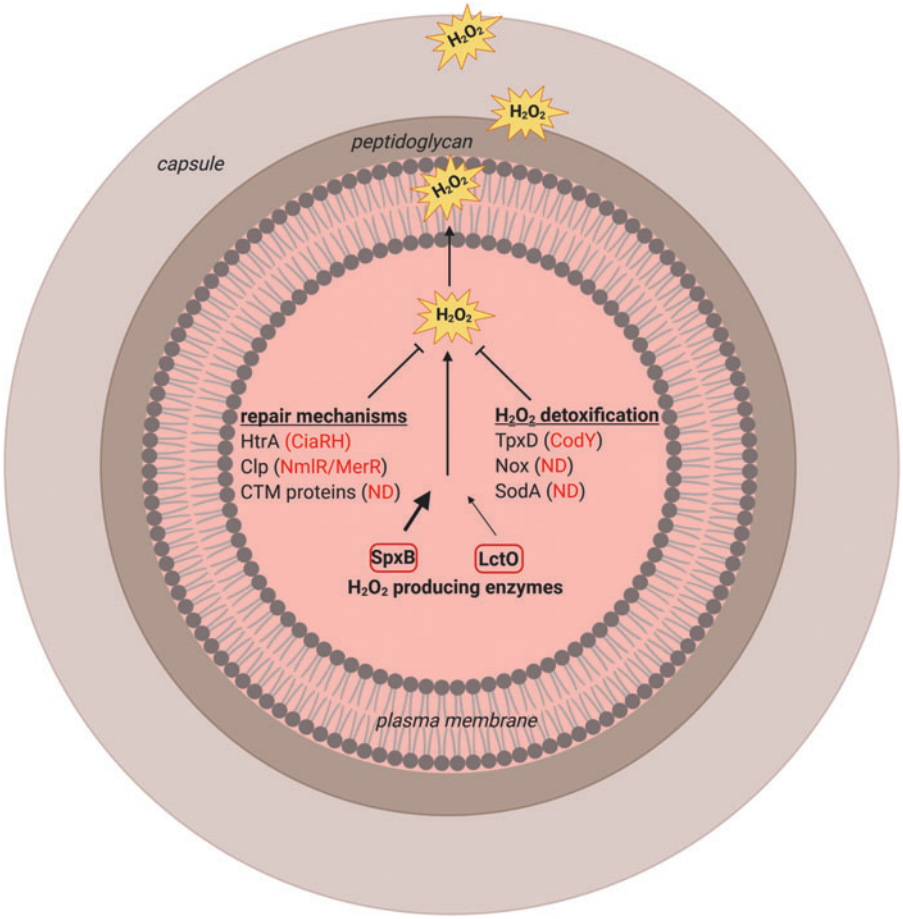

FIG. 1. Mechanisms of pneumococcal defense against H_2O_2. Schematic overview of the three defense mechanisms involved in the resistance against H_2O_2: (i) defense enzymes that directly or indirectly degrade H_2O_2, (ii) repair mechanisms, and (iii) regulators linked to H_2O_2 stress response. H_2O_2, hydrogen peroxide. Color images are available online.

O_2 (73). In prokaryotes, SODs are distinguished by their metal cofactors: Fe^{3+}, Mn^{2+}, Cu^{2+}/Zn^{2+}, and Ni^{2+} (31, 37, 140). Previous studies demonstrated that *E. coli* mutants deficient in both the manganese- and iron-containing SODs are more sensitive to H_2O_2 (21). Furthermore, the expression of MnSOD (SodA) from *S. thermophiles* in bacteria not expressing SODs, such as *L. gasseri* and *L. acidophilus*, provided protection against H_2O_2 stress (16). It seems that SOD protects cells against H_2O_2 by removing $O_2^{\bullet-}$ and preventing the redox cycling of iron.

Although *Spn* contains two types of SODs—MnSOD and FeSOD—only MnSOD (SodA) levels were increased during aerobic growth, and deletion of *SodA* lowered the growth of the bacterium in aerobic conditions. Virulence studies revealed attenuated growth of the *sodA* deletion mutant in intranasally infected mice, with growth rates in the lung and bloodstream comparable with wild type (143). In conclusion, the mechanism by which SOD provides protection of *Spn* against H_2O_2 is indirectly targeted at removing $O_2^{\bullet-}$ and preventing the redox cycling of iron.

A putative gene for *ahpD*, a group of enzymes known to degrade H_2O_2, was detected in *Spn* (92, 100). However, AhpD does not seem to provide H_2O_2 resistance in *Spn*, as its depletion rather increased the resistance of *Spn* against H_2O_2 (100).

Pneumococcal proteins involved in repair of H_2O_2-induced damage. Cellular damage caused by oxidative stress is multilayered and rapid. Therefore, in addition to enzymatic degradation of H_2O_2, immediate repair of damaged proteins and lipids is essential for the survival of the bacterium. *Spn* is equipped with a number of proteins known to be involved in the repair of H_2O_2-induced damage. These proteins include the following: (i) high-temperature requirement A, a serine protease, and chaperone protein, (ii) Clp ATP-dependent proteases, and (iii) CTM proteins consisting of CcdA (putative cytochrome *c*-type biogenesis protein homologue), thioredoxin-like protein family (TlpA), and methionine sulfoxide reductase A/B protein (MsrAB). All of these proteins participate in the recovery from H_2O_2-induced oxidative stress, as their progressive removal leads to an increased susceptibility of *Spn* to H_2O_2 [reviewed in ref. (142)]. Table 1 and Figure 1 summarize the mode of action and the regulators of proteins involved in pneumococcal defense against H_2O_2.

Impact of H_2O_2 on iron homeostasis. Transition of *Spn* from the nasopharynx to the lungs requires adjustment to a variety of environmental conditions, including availability of transition metal ions (98). Iron (Fe^{2+}) potentiates oxidative stress. Expression of Fe^{2+} transport systems and proteins that protect against oxidative stress is regulated by the orphan response regulator RitR. Deletion of *ritR* impaired *Spn* growth in high-Fe^{2+} medium, which could be reversed upon addition of manganese (Mn^{2+}), the latter of which caused a reduction in the amount of H_2O_2 produced by *Spn*.

In *Spn,* excess levels of Zn^{2+} prevent the uptake of Mn^{2+} by irreversibly binding to the extracellular Mn^{2+}-binding protein pneumococcal surface adhesin A (89, 124). As a consequence, *Spn* becomes hypersensitive to oxidative stress, due to a decrease in the activity of MnSOD, which plays an important role in protecting the pathogen against high exogenous H_2O_2 levels (125). It is likely that many of the enzymes involved in resistance to ROS in *Spn* metallate with manganese rather than with iron.

H_2O_2 can interact with ferrous ions (Fe^{2+}) and form a highly reactive hydroxyl radical ($^{\bullet}OH$) through the Fenton reaction, causing DNA damage and increased toxicity to the cells. *Spn* contains only a small number of iron-containing proteins, in contrast to most other bacteria, such as *E. coli*, and thereby avoid poisoning themselves during radical attack. This is consistent with findings showing that H_2O_2-mediated killing of *Spn* is unaffected by iron chelators and appears to be independent of the Fenton reaction (104).

A homologue of DNA-binding protein from starved cells (Dps) and of Dps-like peroxide resistance (Dpr) is present in the pneumococcal genome. Unlike Dps, which protects DNA through direct association, Dpr was found to be an important factor for mediating resistance of oxidative stress caused by H_2O_2 through chelation of free iron, thereby inhibiting the Fenton reaction. Furthermore, a *dpr* mutant has a reduced ability to colonize and is more rapidly cleared from the nasopharynx in a mouse model (65). In *S. suis*, Dpr can bind ferrous ions as well as other divalent cations, such as Cu^{2+}, Mn^{2+}, and Zn^{2+} (52).

Critical Issues: Actions of H_2O_2 on the Host's Lung Cells

Spn-*derived* H_2O_2 *induces the UPR in host cells*

Endoplasmic reticulum (ER) stress, resulting from the accumulation of unfolded proteins, increased protein load, or calcium gradient dysregulation, can trigger the UPR. This fundamental stress response is used by eukaryotic cells to match demand for protein synthesis with the capability to fold proteins within the ER, to maintain cellular homeostasis (148). In lower organisms, such as *Caenorhabditis elegans*, the UPR was demonstrated to participate in the defense against pathogens, since loss of this pathway was shown to induce hypersensitivity to certain pore-forming toxins, but not to other toxic insults (10).

UPR signaling is mediated by three transducers that are inserted into the ER membrane: activating transcription factor 6 (ATF6), protein kinase R (PKR)-like ER kinase (PERK), and inositol-requiring enzyme 1 (IRE1). The ER-resident chaperone immunoglobulin heavy chain-binding protein (BiP) binds to the luminal domain of the ER stress sensors and keeps them in a monomeric inactive state. Accumulation of unfolded proteins leads to release of BiP from the ER stress sensors and induces its subsequent binding to the unfolded proteins (20, 24). PERK and IRE1 become activated by dimerization (or oligomerization) and subsequent phosphorylation (19, 26). Phosphorylated IRE1 activates its own endoribonuclease, which mediates splicing from X-box-binding protein 1 (XBP1) mRNA. Translation of the spliced transcript produces active XBP1 protein that translocates to the nucleus, where it induces the transcription of several ER-resident chaperones and protein-folding enzymes (57, 70).

PERK activation induces phosphorylation of serine 51 of eukaryotic translation initiation factor 2α (eIF2α). As a consequence, translation is inhibited and protein folding stress in the ER is decreased. However, PERK activation also increases the translation of the transcription factor activating transcription factor 4 (ATF4). ATF4 mRNA possesses internal ribosome entry site sequences in its 5′-untranslated regions and as such escapes translational suppression. Upon

entering the nucleus, ATF4 regulates the expression of UPR target genes (20). Dissociation of BiP from ATF6 initiates its translocation to the Golgi compartment where it can be cleaved by two proteases. The activated transcription factor ATF6 then migrates to the nucleus and activates the expression of genes encoding proteins, which enhance ER capacity and function (including BiP) (138, 145).

Although UPR signaling can alleviate ER stress, it can also—when sustained—activate deleterious cell death pathways if homeostasis cannot be restored (99). Recently, activation of the UPR was even suggested to mediate vascular disease (105, 112). Indeed, the p22phox subunit, an essential component of most Noxs, was shown to be a novel target of the UPR transcription factor ATF4 under ER stress conditions, thereby increasing ROS generation from Noxs and promoting pathogenesis of cardiovascular disease. Hence, the UPR plays an important role in physiology, but can also contribute to development of cardiovascular disease. Infection with *Spn* can induce the UPR (6, 70, 93). This was demonstrated by the expansion of ER membranes in the H441 cell line (human lung adenocarcinoma similar to club-like lung epithelial cells) upon infection (75), a common feature observed during the UPR (106).

Secretion of *Spn*-derived H_2O_2 leads to activation of PERK, ATF-6, and IRE1 (Fig. 2). Dimerization and phosphorylation of activated PERK induce phosphorylation of eIF2α leading to inhibition of protein translation and ATF4 modulating expression of target genes (Fig. 2). Activated ATF-6 translocates to the Golgi, where it is cleaved by site-1 protease and site-2 protease. The processed ATF-6 enters the nucleus acting as a transcription factor of target genes. Activation of IRE1 leads to splicing of *xbp1* mRNA, which acts as transcription factor of target genes. IRE1 activation can also lead to regulated Ire1-dependent decay (RIDD of mRNA) or c-Jun N-terminal kinase (JNK) signaling activation. Induction of the UPR slows down ongoing protein synthesis and increases the folding capacity of the ER.

Although a number of bacterial virulence factors, including lipopolysaccharide (LPS) and some pore-forming toxins, such as listeriolysin-O, were shown to induce the UPR (10, 24, 75), infections with *Spn* in H441 cells revealed that H_2O_2, rather than the pore-forming toxin Ply, is the main trigger of UPR, mainly by means of activating the PERK pathway (Fig. 2). The important role of H_2O_2 in the induction of the UPR during pneumococcal infection is documented by the observation that a mutant *Spn* strain lacking pyruvate oxidase

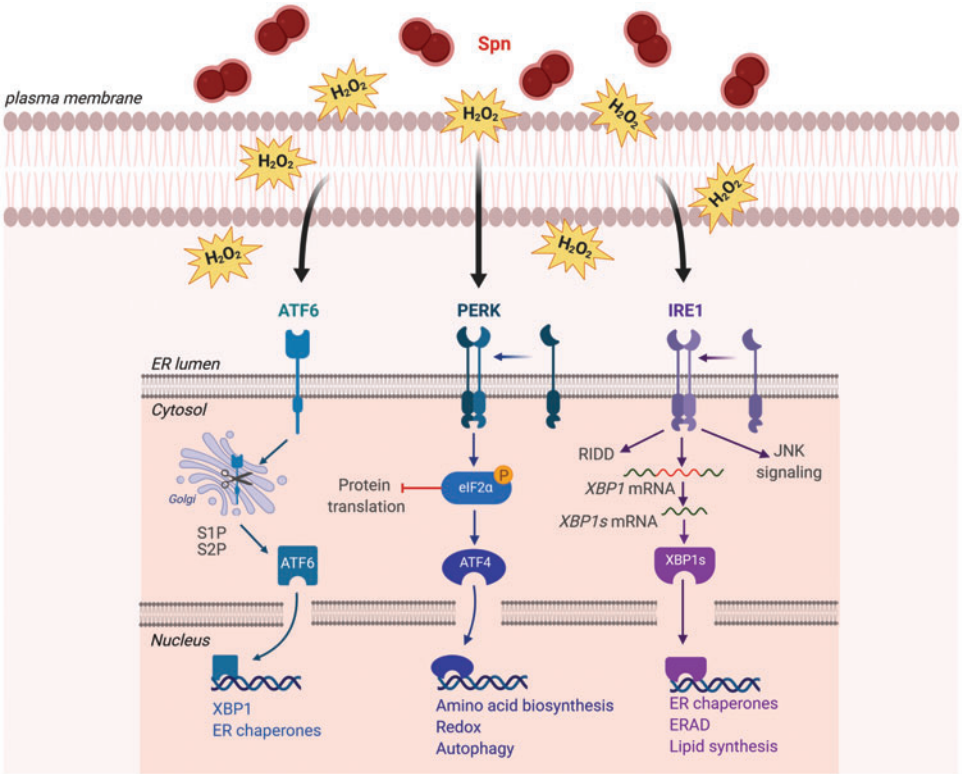

FIG. 2. Actions of *Spn*-derived H_2O_2 on the UPR in host cells. Infection with *Spn* induces the activation of the UPR. Secretion of *Spn*-derived H_2O_2 leads to activation of PERK, ATF-6, and IRE1. Dimerization and phosphorylation of activated PERK induce phosphorylation of eIF2α leading to inhibition of protein translation and ATF4 modulating expression of target genes. Activated ATF-6 translocates to the Golgi, where it is cleaved by S1P and S2P. The processed ATF-6 enters the nucleus acting as a transcription factor of target genes. Activation of IRE1 leads to splicing of *xbp1* mRNA, which acts as transcription factor of target genes. IRE1 activation can also lead to RIDD of mRNA or JNK signaling activation. ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; IRE1, inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; PERK, protein kinase R (PKR)-like ER kinase; RIDD, regulated IRE1-dependend decay; S1P, site-1-protease; S2P, site-1-protease; *Spn*, *Streptococcus pneumonia*; UPR, unfolded protein response; xbp1, X-box-binding protein 1. Color images are available online.

(SpxB), which secretes a very low level of H_2O_2, does not induce UPR in H441 cells (75).

Induction of DNA damage by pneumococcal-secreted H_2O_2

Spn not only generates large amounts of H_2O_2 endogenously (34), but also releases large quantities of H_2O_2 *in vitro* and *in vivo* (42). Host cells typically trigger an antioxidant stress response during *Spn* infection, to prevent oxidative injury. This protective response occurs by inducing the production of the transcription factor nuclear factor erythroid 2-related factor 2, which plays a crucial role in the transcriptional activation of antioxidant enzymes and ROS scavengers. These include heme oxygenase 1, NADPH dehydrogenase, and SOD (73, 83).

At excessive concentrations, H_2O_2 can, however, induce toxic DNA double-strand breaks, which precede apoptosis, as was shown using cultures of the human A549 cell line (a model for type II pneumocytes) exposed to three serotypes of *Spn*. Catalase reduced the frequency of DNA damage-positive cells in these studies by 50% or more (109).

Another consequence of bacterial H_2O_2 release is an increased production of mitochondrial ROS in the host cells, as observed during infection with wild-type *Spn*, with a Ply-negative mutant strain (Δ*ply*), but not with a SpxB-negative *Spn* strain (63, 75) (Fig. 3). *Spn*-secreted H_2O_2 can also cause oxidative damage to mitochondrial DNA (mtDNA) and lead to the release of mtDNA into the cytoplasm, which in turn induces type I interferon expression in the A549 cell line, involving the stimulator of interferon gene signaling pathway (46) (Fig. 3).

Pneumococcal H_2O_2 affects inflammation in host cells

Apart from activating mtROS generation, DNA damage, and UPR in host cells, H_2O_2 secreted by *Spn* can also induce activation of all three mitogen-associated protein kinase subfamilies, that is, p38, JNK, and ERK, and cause nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) translocation and transcription of proinflammatory chemokines and cytokines (interleukin [IL]-8 and IL-23a) (Fig. 3) (9, 75). Canonical activation of the transcriptional regulatory factor NF-κB protein complex involves phosphorylation-induced proteolysis of the inhibitory protein IκBα, which is bound to NF-κB subunits RelA and p50 by IκB kinase. Proteolysis of IκBα leads to subsequent translocation of RelA/p50 protein complex to the nucleus (Fig. 3).

The ability of lung parenchymal cells to contribute to proinflammatory cytokine production has also been demonstrated in response to other pathogens, including *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *H. influenza* (22, 69, 110, 114). A mutation in pyruvate oxidase (SpxB), causing reduced H_2O_2 secretion, was shown to diminish adhesive properties of *Spn* to type II pneumocytes and capillary endothelial cells, which may contribute to diminished virulence *in vivo* (118).

Despite activating certain inflammatory pathways, pneumococcal-secreted H_2O_2 does not necessarily activate the host's immune defense. By contrast, recent results indicate that H_2O_2 released by *Spn* inhibits inflammasomes, key components of the innate immune system, as such further contributing to virulence and to colonization of the host (36). Thereby, H_2O_2, which is secreted in millimolar quantities by some *Spn* strains,

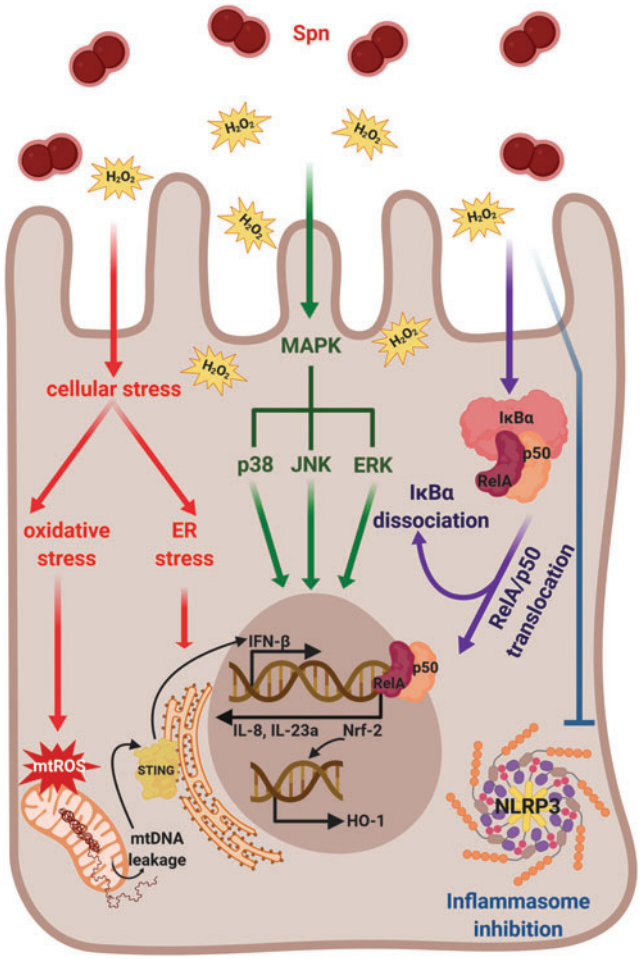

FIG. 3. H_2O_2-induced responses in the host cell. *Spn* produces large amounts of H_2O_2, which induce a plethora of host cell responses. This includes oxidative and ER stress (*red*), activation of all three MAPK subfamilies (*green*), translocation of NF-κB-complex into the nucleus (*violet*), and inflammasome inhibition (*blue*). Oxidative stress leads to production of mtROS and release of mtDNA and subsequent STING-dependent type I IFN (IFN-β) expression. Translocation of NF-κB-complex into the nucleus leads to expression of proinflammatory chemokines and cytokines (such as IL-8 and IL23a) and H_2O_2-dependent production of Nrf-2, which induces expression of HO-1. HO-1, heme oxygenase 1; IFN, interferon; IL, interleukin; MAPK, mitogen-activated protein kinase; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STING, stimulator of interferon genes. Color images are available online.

has the capacity to counteract the NLRP3 inflammasome-activating effect of Ply, the main virulence factor of *Spn* (91). On the contrary, pneumococcus-derived H_2O_2 was shown to directly promote aggregation and activation of platelets, cells orchestrating the systemic inflammatory response (1).

Dose-dependent effects of H_2O_2 on vectorial Na^+ transport in alveolar epithelial cells

The level of fluid in alveoli represents a critical balance between the rate of fluid movement from pulmonary

capillaries and lung interstitium across the alveolar barrier into the alveolar space and the transport of salt and water out of the alveoli. Vectorial Na^+ transport out of the alveoli by the apically expressed epithelial sodium channel (ENaC) and the basolaterally expressed Na^+–K^+ pump in flat type I (cover 90% of alveolar surface) and cuboidal type II alveolar epithelial cells (cover 5% of alveolar surface) is the major mechanism to maintain an optimal level of airway and alveolar surface liquid (35, 86, 87, 116, 126, 127).

ENaC, whose activity is defined by its surface expression (*N*) and its open probability time (*Po*), is critically involved in alveolar fluid clearance (AFC) (35, 87). Indeed, genetic deletion of the crucial α subunit in neonatal mice prevents them from clearing fluid from their lungs, leading to respiratory distress and death shortly after birth (66). The observation that single-nucleotide polymorphisms rs4149570 and rs7956915 of ENaC-α are associated with neonatal respiratory distress syndrome and lung fluid absorption disorders indicates that ENaC-α (*SCNN1A*) also has a significant role in fluid clearance in man (72). When these mechanisms become impaired, as is the case in severe pneumonia, alveolar flooding occurs, which can precipitate a lethal hypoxemia by impairing gas exchange.

Concentrations of H_2O_2 in the alveolar space in healthy individuals were estimated to be in the micromolar to tens of micromolar range (25). Physiological concentrations of endogenous, dual oxidases 1/2-mediated and exogenous H_2O_2 were shown to increase ENaC activity (39, 60, 82). This occurs at least partially through a reduction in ubiquitination of the ENaC-α subunit, which blunts the subunit's degradation and thus increases its surface expression *N* (33). At physiological concentrations, H_2O_2 also activates PI3-kinases that produce the anionic phospholipids, phosphatidylinositol-4,5-bisphosphate and phosphatidylinositol-3,4,5-trisphosphate, which were shown to increase the amount of time that ENaC spends in an open state, transporting Na^+ (*Po*) (60, 67, 108, 147). Physiologic levels of H_2O_2 also stimulate the Na^+–K^+ pump in alveolar epithelial cells (29, 49, 127).

However, during pneumococcal pneumonia, near millimolar concentrations of H_2O_2 can be secreted by *Spn* in the alveolar space, in addition to the H_2O_2 generated by activated neutrophils and alveolar macrophages (Fig. 4). These high levels suppress ENaC-α transcription, in an ERK-dependent manner, and can inhibit the Na^+–K^+ pump (29, 49, 127, 131, 141). As a consequence, vectorial Na^+ transport is impaired and alveolar flooding can occur, which can precipitate a

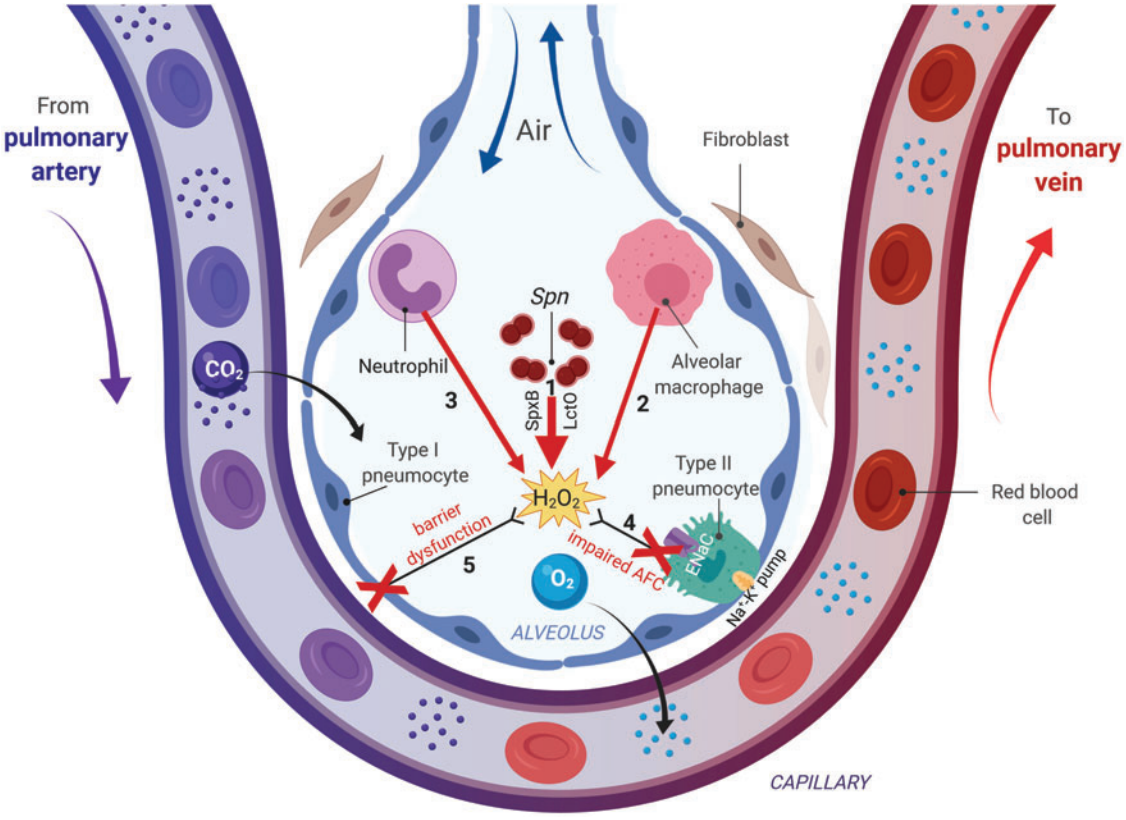

FIG. 4. The complex actions of H_2O_2 in alveoli during pneumococcal pneumonia. *Spn* will migrate into the lower respiratory tract, and since it lacks catalase will generate millimolar levels of H_2O_2, through the actions of pyruvate oxidase (SpxB) and lactate oxidase (LctO), which will diffuse into the alveolar space (1). Moreover, an early neutrophil-mediated and a later macrophage-derived generation and secretion of μmolar levels of H_2O_2 will occur (2, 3). High ROS levels in the alveolar space will promote alveolar endothelial barrier function (4) and will impair AFC (5), which is mainly mediated through vectorial sodium transport, involving the apically expressed ENaC and the basolateral Na^+-K^+ pump in type II pneumocytes. AFC, alveolar fluid clearance; ENaC, epithelial sodium channel; ROS, reactive oxygen species. Color images are available online.

lethal hypoxemia by impairing gas exchange (Fig. 4). *Spn* release of Ply can aggravate this further, since this toxin has also been shown to impair ENaC activity (77).

Impact of H_2O_2 on alveolar–capillary barrier function

A tight barrier structure of only 0.3 μm thickness separates capillary blood from alveolar gas and is a crucial interface for efficient gas exchange (133, 135). A disruption of the alveolar–capillary barrier, if not resolved, can result in alveolar flooding and poor alveolar gas exchange, both of which lead to hypoxia and hypercapnia, hallmarks of ARDS (127). During pneumococcal pneumonia, high H_2O_2 levels accumulate in the alveolar space, originating mainly from *Spn* secretion, and to a lesser extent also from inflammatory neutrophils and alveolar macrophages, which first generate superoxide from Nox-2, which is then further converted into H_2O_2 by SOD.

An important role of H_2O_2 in the pathogenesis of acute lung injury was first recognized by the observation that both catalase and SOD protected sheep lungs from air emboli-induced increased vascular permeability (42). Moreover, intravascular H_2O_2 challenge in intact lungs was shown to cause both capillary endothelial and alveolar epithelial barrier dysfunction, characterized by increased actin stress fiber formation, intercellular gaps, and intracellular Ca^{2+} levels. These effects on barrier function could be partially inhibited by increasing cAMP or cGMP levels (102, 115, 117).

H_2O_2 can mediate alveolar epithelial–endothelial paracrine signaling. As such, increased H_2O_2 levels in the alveolar space and pneumocytes can contribute to capillary endothelial barrier dysfunction (63). Recently, an important role for transient receptor potential (TRP) channels was suggested in the actions of ROS. This family of cation channels depolarizes the membrane potential and regulates intracellular concentrations of cations such as Ca^{2+}, the latter of which is known to regulate barrier function (113). Several TRP channels, including transient receptor potential melastatin 2 (TRPM2), transient receptor potential vanilloid-4 (TRPV4), and transient receptor potential channel 6 (TRPC6), are redox sensors that can be activated by H_2O_2 (5, 32, 84, 95). Micromolar levels of H_2O_2 were shown to stimulate ADP ribose formation in human pulmonary artery endothelial cells and to activate the Ca^{2+}-permeable and oxidant-activated TRPM2 channel. H_2O_2-mediated Ca^{2+} entry and diminished transendothelial electrical resistance in these cells were blunted by TRPM2 siRNA depletion or by a neutralizing antibody (59). TRPM2 is also expressed in alveolar epithelial cells, and TRPM2 knockout mice were shown to be less susceptible to bleomycin-induced lung inflammation, which is known to be accompanied by increased H_2O_2 levels (144).

Phosphorylation of TRPV4 by the Src family kinase Fyn, which is tethered to the cell membrane by the fatty acid transporter CD36, was recently shown to significantly contribute to H_2O_2-induced Ca^{2+} influx in lung microvascular endothelial cells (120). Proposed molecular mechanisms of TRPV4-mediated edema formation include Ca^{2+}-induced activation of myosin light-chain kinase in endothelial cells (11) and the opening of Ca^{2+}-activated K^+ channels (KCa3.1) in epithelial cells, which induce hyperpolarization (130).

Finally, TRPC6 was shown to be critically involved in the regulation of pulmonary vascular permeability and lung edema formation during LPS or ischemia/reperfusion-induced acute lung injury (134).

Clinical significance of endogenous H_2O_2 generation during pneumococcal pneumonia

The *SpxB* gene found in nearly all *Spn* strains encodes pyruvate oxidase, an enzyme that converts pyruvate to acetyl phosphate and generates near suicidal levels of H_2O_2. These high levels of endogenous H_2O_2 would normally cause bacterial death in the absence of catalase, yet *Spn* is able to resist them and to even use them as a factor to outcompete *S. aureus* in the nose and to foster colonization in the host. As indicated above, a general strategy used by *Spn* and lactic-acid bacteria to avoid the toxic effects of the Fenton reaction is replacing iron with manganese. Also, the production of high levels of ferritin-binding proteins keeps free-iron levels low enough to suppress damage to DNA.

The complex role of H_2O_2 in pneumococcal-infected patients was clearly documented by recent observations with clinical isolates of serotype 1 belonging to 2 major clonal complexes (CCs), that is, CC228, associated with low mortality, and CC217, associated with high mortality (15, 121). Mutations in *SpxB* resulted in larger colony variants unable to produce endogenous H_2O_2. Intravenous challenge of mice with these mutants revealed that early macrophage-mediated clearance was lower than that of wild-type bacteria, and resulted in a higher bacterial load (121). Nevertheless these mutants were less efficient in the colonization process when compared with the wild-type strain. Thus, a requirement for H_2O_2 in the infection process varies as the bacteria moves from niches that are oxygen rich to those that have low oxygen tension.

Alcoholics are at increased risk for developing pneumococcal pneumonia and a role for pneumococcal H_2O_2 for this predilection has also been postulated. The *Spn* type 2 D39 strain, which is ethanol-tolerant due to increased alcohol dehydrogenase E (*adhE*) expression, exhibits increased H_2O_2 generation. Thus, ethanol-fed mice were more susceptible to infection with the D39 wild-type bacteria than with the Δ*adhE* strain (80).

Future Directions

Pulmonary permeability edema as a consequence of the ARDS is a main cause of morbidity and mortality of pneumococcal pneumonia. In the absence of proven pharmacologic treatments, clinicians have to rely on ventilation strategies to deliver oxygen to patients, which can sometimes make the situation worse if the ventilation pressure is too high and causes ventilator-induced lung injury. Permeability edema is characterized by hyperpermeability of the alveolar–capillary barrier, combined with a deficiency to clear liquid from the alveolar space. Vectorial Na^+ transport through the apically expressed ENaC and the basolateral Na^+–K^+ pump is crucial for AFC. ARDS patients with an impaired capacity to clear alveolar fluid were shown to be at significantly higher risk to die (132).

During pneumococcal pneumonia, the pathogen can generate millimolar amounts of H_2O_2 that can rapidly diffuse through cell membranes and accumulate in the alveolar space. Whereas endogenous H_2O_2 clearly represents a colonization advantage to the pathogen, exogenous H_2O_2 in the $\sim 10\ \mu M$ range generated by the Nox-2/MPO or Nox2/SOD

pathways in neutrophils and alveolar macrophages promotes bacterial clearance. Although the detailed mechanism by which the catalase-negative pneumococci can withstand high concentrations of endogenous and phagocyte-derived exogenous H_2O_2 is incompletely understood, several well-defined enzymatic pathways in the pathogen and in phagocytic cells of the host are involved, as summarized in Figure 5.

Excessively high levels of exogenous H_2O_2 in the alveolar space during pneumococcal pneumonia can be damaging to the pathogen, they also have effects on host cells of the alveolar space. Because H_2O_2 is membrane permeant, it can promote DNA damage and apoptosis, induce the UPR and blunt inflammasome-mediated host defense, and increase inflammation. In particular, type 1 and 2 alveolar epithelial cells and the microvascular endothelial cells make up the alveolar–capillary barrier in the lungs affected by high H_2O_2 concentrations. As a consequence of reduced subunit expression, impaired Na^+-transport by ENaC and the Na^+–K^+ pump in AT1/2 cells will lead to a dysfunctional AFC. Moreover, impaired barrier function in both the epithelial and endothelial compartments will promote fluid, protein, and cell accumulation in the alveolar space.

Future therapeutic approaches for pneumococcal pneumonia should potentially aim at reducing the harmful effects of ROS in lung cells, without impairing host defense. As findings with SpxB mutants have indicated, strategies to blunt endogenous H_2O_2 generation in the pathogen seem challenging. However, agents able to impair endogenous H_2O_2 generation or promote their downstream effects on iron homeostasis could decrease the harmful effects of the pathogen and improve macrophage-mediated host defense.

In view of their role in barrier function, TRP channel inhibitors could represent attractive therapeutic candidates to counteract the actions of high levels of H_2O_2 during pneumococcal pneumonia. However, their rather ubiquitous expression and the multitude of their functions (regulation of the inflammatory response, pulmonary vasomotor control, and systemic blood pressure) may render also this approach challenging.

As such, therapeutic candidates that reduce the deleterious effects of oxidative stress on barrier function and AFC specifically in the host cells, without impairing antipneumococcal host defense, might hold the key to developing a successful pharmacological approach to the potentially lethal permeability edema associated with severe pneumonia.

The alpha-tocopherol form of vitamin E was shown to boost elastase activity of human neutrophils as well as their ability to kill *Spn* (13). Especially noteworthy are promising preclinical data with the potent antioxidant ascorbic acid (vitamin C), which in preclinical studies was shown to attenuate systemic inflammation and blunt sepsis-induced coagulopathy and vascular injury (40, 41, 43). In a recent phase 2 clinical trial in severely ill patients with sepsis and ARDS, high-dose intravenous administration of vitamin C did not significantly improve the primary endpoint, that is, modified Sequential Organ Failure Assessment (mSOFA) score at 96 h (44). Yet, taking into account mSOFA scores from patients who died during the trial, the mSOFA score at 96 h was significantly lower in patients on the test drug than in the placebo group (45).

A tumor necrosis factor (TNF)-derived peptide—the TIP peptide (a.k.a. AP301, solnatide), which mimics the lectin-

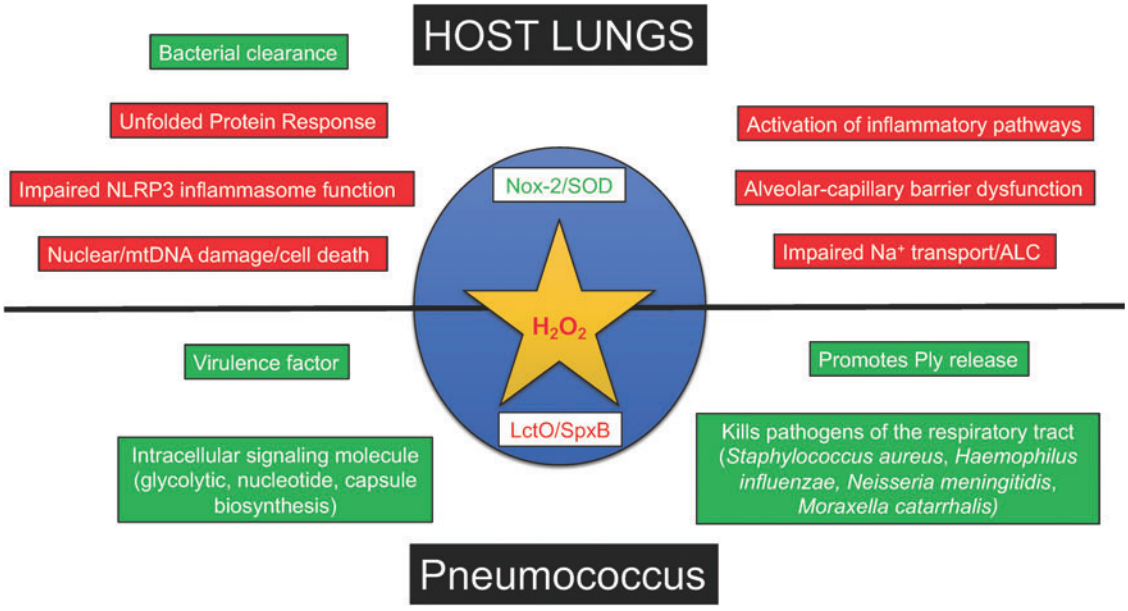

FIG. 5. Overview of positive (*green*) and negative (*red*) actions of endogenous and secreted H_2O_2 in the pathogen and in the host lung during pneumococcal pneumonia. In *Spn*, endogenous H_2O_2 promotes nucleotide, glycolytic, and capsule biosynthesis and increases Ply secretion. Secreted H_2O_2 kills competing pathogens in the respiratory tract and serves as a virulence factor, since it reduces NLRP3 inflammasome activation in the host. In the alveolar space, *Spn*-secreted H_2O_2 can induce activation of the UPR and cause DNA damage, which can precede apoptosis. Moreover, H_2O_2 can impair the alveolar–capillary barrier, induce inflammation, and blunt vectorial Na^+ transport, crucial for AFC in pneumocytes. All of these actions of H_2O_2 on the host lung cells can promote the development of pulmonary edema during pneumococcal pneumonia. Ply, pneumolysin. Color images are available online.

like domain of the cytokine and directly binds to the α subunit of ENaC, the latter of which is expressed in both epithelial and endothelial lung cells (27, 28), was shown to significantly reduce oxidative stress in pulmonary artery endothelial cells under hypoxia/reoxygenation and in transplanted rat lungs (55, 56). The peptide significantly blunted bacterial toxin-induced capillary leak and pulmonary edema in mice, rats, rabbits, and pigs and was moreover shown in a phase 2 clinical trial to significantly reduce extravascular lung water index in acute lung injury patients (a measure for edema) (68). Interestingly the lectin-like domain of TNF was shown not to interfere with antibacterial activities of the cytokine in a murine model of septic peritonitis (76).

In conclusion, H_2O_2 plays a complex role during pneumococcal pneumonia. On the one hand, its generation by the host's phagocytes can reduce bacterial burden. On the other hand, excessive endogenous generation of H_2O_2 by the pathogen provides a means to outcompete other pathogens inhabiting the respiratory tract and to increase resistance to exogenous levels of H_2O_2. Moreover, excessive levels of ROS can significantly impair host defense and foster the formation of permeability edema in the lungs, through the impairment of alveolar–capillary barrier function and AFC capacity.

Strategies that specifically blunt H_2O_2's deleterious actions on the host and promote pathogen susceptibility could represent a promising approach to reduce the high mortality associated with pneumococcal pneumonia.

Acknowledgments

Figures 1–4 were prepared using BioRender. The authors acknowledge funding institutions.

Author Disclosure Statement

R.L. is inventor on patents related to the use of the TIP peptide in ARDS. The other authors have no conflict of interest.

Funding Information

The study was funded by the National Institutes of Health HL134934 (Y.S.), HL125926 (D.F.), DK110409 (D.C.E.), and HL138410 (R.L.), by the Lungen- und Atmungsstiftung Bern, Switzerland (J.H., R.L.), and by the German Research Foundation SFB-TR 84 ''Innate Immunity of the Lung: Mechanisms of Pathogen Attack and Host Defense in Pneumonia'' (M.A.M. and T.C.).

References

1. Anderson R and Feldman C. Review manuscript: mechanisms of platelet activation by the pneumococcus and the role of platelets in community-acquired pneumonia. *J Infect* 75: 473–485, 2017.
2. Anderson R, Steel HC, Cockeran R, von Gottberg A, de Gouveia L, Klugman KP, Mitchell TJ, and Feldman C. Comparison of the effects of macrolides, amoxicillin, ceftriaxone, doxycycline, tobramycin and fluoroquinolones, on the production of pneumolysin by *Streptococcus pneumoniae* in vitro. *J Antimicrob Chemother* 60: 1155–1158, 2007.
3. Auzat I, Chapuy-Regaud S, Le Bras G, Dos Santos D, Ogunniyi AD, Le Thomas I, Garel JR, Paton JC, and Trombe MC. The NADH oxidase of *Streptococcus pneumoniae*: its involvement in competence and virulence. *Mol Microbiol* 34: 1018–1028, 1999.
4. Balachandran P, Hollingshead SK, Paton JC, and Briles DE. The autolytic enzyme LytA of *Streptococcus pneumoniae* is not responsible for releasing pneumolysin. *J Bacteriol* 183: 3108–3116, 2001.
5. Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E, Eidam HS, Ye G, Willette RN, Thorneloe KS, Bradshaw HB, Matalon S, and Jordt SE. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 307: L158–L172, 2014.
6. Baruch M, Hertzog BB, Ravins M, Anand A, Cheng CY, Biswas D, Tirosh B, and Hanski E. Induction of endoplasmic reticulum stress and unfolded protein response constitutes a pathogenic strategy of group A *Streptococcus*. *Front Cell Infect Microbiol* 4: 105, 2014.
7. Benisty R, Cohen AY, Feldman A, Cohen Z, and Porat N. Endogenous H_2O_2 produced by *Streptococcus pneumoniae* controls FabF activity. *Biochim Biophys Acta* 1801: 1098–1104, 2010.
8. Berger S, Goekeri C, Gupta SK, Vera J, Dietert K, Behrendt U, Lienau J, Wienhold SM, Gruber AD, Suttorp N, Witzenrath M, and Nouailles G. Delay in antibiotic therapy results in fatal disease outcome in murine pneumococcal pneumonia. *Crit Care* 22: 287, 2018.
9. Bhatia M, Zemans RL, and Jeyaseelan S. Role of chemokines in the pathogenesis of acute lung injury. *Am J Respir Cell Mol Biol* 46: 566–572, 2012.
10. Bischof LJ, Kao CY, Los FC, Gonzalez MR, Shen Z, Briggs SP, van der Goot FG, and Aroian RV. Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. *PLoS Pathog* 4: e1000176, 2008.
11. Bogatcheva NV, Zemskova MA, Poirier C, Mirzapoiazova T, Kolosova I, Bresnick AR, and Verin AD. The suppression of myosin light chain (MLC) phosphorylation during the response to lipopolysaccharide (LPS): beneficial or detrimental to endothelial barrier? *J Cell Physiol* 226: 3132–3146, 2011.
12. Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, van Werkhoven CH, van Deursen AM, Sanders EA, Verheij TJ, Patton M, McDonough A, Moradoghli-Haftvani A, Smith H, Mellelieu T, Pride MW, Crowther G, Schmoele-Thoma B, Scott DA, Jansen KU, Lobatto R, Oosterman B, Visser N, Caspers E, Smorenburg A, Emini EA, Gruber WC, and Grobbee DE. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 372: 1114–1125, 2015.
13. Bou Ghanem EN, Lee JN, Joma BH, Meydani SN, Leong JM, and Panda A. The alpha-tocopherol form of vitamin E boosts elastase activity of human PMNs and their ability to kill *Streptococcus pneumoniae*. *Front Cell Infect Microbiol* 7: 161, 2017.
14. Brown AO, Millett ER, Quint JK, and Orihuela CJ. Cardiotoxicity during invasive pneumococcal disease. *Am J Respir Crit Care Med* 191: 739–745, 2015.
15. Brueggemann AB and Spratt BG. Geographic distribution and clonal diversity of *Streptococcus pneumoniae* serotype 1 isolates. *J Clin Microbiol* 41: 4966–4970, 2003.
16. Bruno-Bárcena JM, Andrus JM, Libby SL, Klaenhammer TR, and Hassan HM. Expression of a heterologous man-

ganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. *Appl Environ Microbiol* 70: 4702–4710, 2004.
17. Bryant JC, Dabbs RC, Oswalt KL, Brown LR, Rosch JW, Seo KS, Donaldson JR, McDaniel LS, and Thornton JA. Pyruvate oxidase of *Streptococcus pneumoniae* contributes to pneumolysin release. *BMC Microbiol* 16: 271, 2016.
18. Carlioz A and Touati D. Isolation of superoxide dismutase mutants in *Escherichia coli:* is superoxide dismutase necessary for aerobic life? *EMBO J* 5: 623–630, 1986.
19. Carrara M, Prischi F, Nowak PR, and Ali MM. Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling. *EMBO J* 34: 1589–1600, 2015.
20. Chaudhari N, Talwar P, Parimisetty A, Lefebvre d'Hellencourt C, and Ravanan P. A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. *Front Cell Neurosci* 8: 213, 2014.
21. Chiang SM and Schellhorn HE. Regulators of oxidative stress response genes in *Escherichia coli* and their functional conservation in bacteria. *Arch Biochem Biophys* 525: 161–169, 2012.
22. Clemans DL, Bauer RJ, Hanson JA, Hobbs MV, St Geme, JW, 3rd, Marrs CF, and Gilsdorfv JR. Induction of proinflammatory cytokines from human respiratory epithelial cells after stimulation by non-typeable *Haemophilus influenzae*. *Infect Immun* 68: 4430–4440, 2000.
23. Cochi SE, Kempker JA, Annangi S, Kramer MR, and Martin GS. Mortality trends of acute respiratory distress syndrome in the United States from 1999 to 2013. *Ann Am Thorac Soc* 13: 1742–1751, 2016.
24. Coope A, Milanski M, Arruda AP, Ignacio-Souza LM, Saad MJ, Anhê GF, and Velloso LA. Chaperone insufficiency links TLR4 protein signaling to endoplasmic reticulum stress. *J Biol Chem* 287: 15580–15589, 2012.
25. Corradi M, Pignatti P, Brunetti G, Goldoni M, Caglieri A, Nava S, Moscato G, and Balbi B. Comparison between exhaled and bronchoalveolar lavage levels of hydrogen peroxide in patients with diffuse interstitial lung diseases. *Acta Biomed* 79: 73–78, 2008.
26. Cui W, Li J, Ron D, and Sha B. The structure of the PERK kinase domain suggests the mechanism for its activation. *Acta Crystallogr D Biol Crystallogr* 67: 423–428, 2011.
27. Czikora I, Alli A, Bao HF, Kaftan D, Sridhar S, Apell HJ, Gorshkov B, White R, Zimmermann A, Wendel A, Pauly-Evers M, Hamacher J, Garcia-Gabay I, Fischer B, Verin A, Bagi Z, Pittet JF, Shabbir W, Lemmens-Gruber R, Chakraborty T, Lazrak A, Matthay MA, Eaton DC, and Lucas R. A novel tumor necrosis factor-mediated mechanism of direct epithelial sodium channel activation. *Am J Respir Crit Care Med* 190: 522–553, 2014.
28. Czikora I, Alli AA, Sridhar S, Matthay MA, Pillich H, Hudel M, Berisha B, Gorshkov B, Romero MJ, Gonzales J, Wu G, Huo Y, Su Y, Verin AD, Fulton D, Chakraborty T, Eaton DC, and Lucas R. Epithelial sodium channel-α mediates the protective effect of the TNF-derived TIP peptide in pneumolysin-induced endothelial barrier dysfunction. *Front Immunol* 8: 842, 2017.
29. Dazy AC, Auger F, Bailbé D, Blouquit S, Lombet A, and Marano F. The toxicity of H_2O_2 on the ionic homeostasis of airway epithelial cells in vitro. *Toxicol In Vitro* 17: 575–580, 2003.
30. Dela Cruz CS, Wunderink RG, Christiani DC, Cormier SA, Crothers K, Doerschuk CM, Evans SE, Goldstein DR, Khatri P, Kobzik L, Kolls JK, Levy BD, Metersky ML, Niederman MS, Nusrat R, OrihuelaCJ, Peyrani P, Prince AS, Ramírez JA, Ridge KM, Sethi S, Suratt BT, Sznajder JI, Tsalik EL, Walkey AJ, Yende S, Aggarwal NR, Caler EV, and Mizgerd JP. Future research directions in pneumonia. NHLBI working group report. *Am J Respir Crit Care Med* 198: 256–263, 2018.
31. Desideri A and Falconi M. Prokaryotic Cu,Zn superoxide dismutases. *Biochem Soc Trans* 31: 1322–1325, 2003.
32. Dietrich A, Steinritz D, and Gudermann T. Transient receptor potential (TRP) channels as molecular targets in lung toxicology and associated diseases. *Cell Calcium* 67: 123–137, 2017.
33. Downs CA, Kumar A, Kreiner LH, Johnson NM, and Helms MN. H_2O_2 regulates lung epithelial sodium channel (ENaC) via ubiquitin-like protein Nedd8. *J Biol Chem* 288: 8136–8145, 2013.
34. Duane P, Rubins JB, Weisel H, and Janoff E. Identification of hydrogen peroxide as a *Streptococcus pneumoniae* toxin for rat alveolar epithelial cells. *Infect Immun* 61: 4392–4397, 1993.
35. Eaton DC, Helms MN, Koval M, Bao HF, and Jain L. The contribution of epithelial sodium channels to alveolar function in health and disease. *Annu Rev Physiol* 71: 403–423, 2009.
36. Erttmann SF and Gekara NO. Hydrogen peroxide release by bacteria suppresses inflammasome-dependent innate immunity. *Nat Commun* 10: 3493, 2019.
37. Fee JA. Regulation of sod genes in *Escherichia coli*: relevance to superoxide dismutase function. *Mol Microbiol* 5: 2599–2610, 1991.
38. Feldman C and Richards G. Appropriate antibiotic management of bacterial lower respiratory tract infections. *F1000Res* 7: F1000, 2018.
39. Fischer H. Mechanisms and function of DUOX in epithelia of the lung. *Antioxid Redox Signal* 11: 2453–2465, 2009.
40. Fisher BJ, Kraskauskas D, Martin EJ, Farkas D, Wegelin JA, Brophy D, Ward KR, Voelkel NF, Fowler AA, 3rd, and Natarajan R. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am J Physiol Lung Cell Mol Physiol*. 303:L20–L32, 2012.
41. Fisher BJ, Seropian IM, Kraskauskas D, Thakkar JN, Voelkel NF, Fowler AA, 3rd, and Natarajan R. Ascorbic acid attenuates lipopolysaccharide induced acute lung injury. *Crit Care Med.* 39: 1454–1460, 2011.
42. Flick MR, Milligan SA, Hoeffel JM, and Goldstein IM. Catalase prevents increased lung vascular permeability during air emboli in unanesthetized sheep. *J Appl Physiol* 64: 929–935, 1988.
43. Fowler AA, 3rd, Fisher BJ and Kashiouris MG. Vitamin C for sepsis and acute respiratory failure-reply. *JAMA* 25; 323: 792–793, 2020.
44. Fowler AA, 3rd, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, Farthing CA, Larus TL, Martin E, Brophy DF, Gupta S, Fisher BJ, and Natarajan R. Medical respiratory intensive care unit nursing. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J Transl Med*. 12: 32, 2014.
45. Fowler AA, 3rd, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, Fisher B, Thacker LR, 2nd, Natarajan R, Brophy DF, Sculthorpe R, Nanchal R, Syed A, Sturgill J, Martin GS, Sevransky J, Kashiouris M, Hamman S, Egan KF, Hastings A, Spencer W, Tench S, Mehkri O, Bindas J,

Duggal A, Graf J, Zellner S, Yanny L, McPolin C, Hollrith T, Kramer D, Ojielo C, Damm T, Cassity E, Wieliczko A, and Halquist M. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. *JAMA* 322: 1261–1270, 2019.

46. Gao Y, Xu W, Dou X, Wang H, Zhang X, Yang S, Liao H, Hu X, and Wang H. Mitochondrial DNA leakage caused by *Streptococcus pneumoniae* hydrogen peroxide promotes type I IFN expression in lung cells. Front Microbiol 10: 630, 2019.
47. GBD 2015 LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and etiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis* 17: 1133–1161, 2017.
48. Gonzales JN, Lucas R, and Verin AD. The acute respiratory distress syndrome: mechanisms and perspective therapeutic approaches. *Austin J Vasc Med* 2: pii: 1009, 2015.
49. González-Flecha B, Evelson P, Ridge K, and Sznajder JI. Hydrogen peroxide increases Na+/K(+)-ATPase function in alveolar type II cells. *Biochim Biophys Acta* 1290: 46–52, 1996.
50. Grousd JA, Rich HE, and Alcorn JF. Host-pathogen interactions in gram-positive bacterial pneumonia. *Clin Microbiol Rev* 32: e00107-18, 2019.
51. Grudzinska FS, Brodlie M, Scholefield BR, Jackson T, Scott A, Thickett DR, and Sapey E. Neutrophils in community-acquired pneumonia: parallels in dysfunction at the extremes of age. *Thorax* 75 [Epub ahead of print]; DOI: 10.1136/thoraxjnl-2018-212826, 2018.
52. Haikarainen T, Thanassoulas A, Stavros P, Nounesis G, Haataja S, and Papageorgiou AC. Structural and thermodynamic characterization of metal ion binding in *Streptococcus suis* Dpr. *J Mol Biol* 405: 448–460, 2011.
53. Hajaj B, Yesilkaya H, Benisty R, David M, Andrew PW, and Porat N. Thiol peroxidase is an important component of *Streptococcus pneumoniae* in oxygenated environments. *Infect Immun* 80: 4333–4343, 2012.
54. Hajaj B, Yesilkaya H, Shafeeq S, Zhi X, Benisty R, Tchalah S, Kuipers OP, and Porat N. CodY regulates thiol peroxidase expression as part of the pneumococcal defense mechanism against H_2O_2 stress. *Front Cell Infect Microbiol* 7: 210, 2017.
55. Hamacher J, Hadizamani Y, Borgmann M, Mohaupt M, Männel DN, Moehrlen U, Lucas R, and Stammberger U. Cytokine-ion channel interactions in pulmonary inflammation. *Front Immunol* 8: 1644, 2018.
56. Hamacher J, Stammberger U, Roux J, Kumar S, Yang G, Xiong C, Schmid RA, Fakin RM, Chakraborty T, Hossain HM, Pittet JF, Wendel A, Black SM, and Lucas R. The lectin-like domain of tumor necrosis factor improves lung function after rat lung transplantation-potential role for a reduction in reactive oxygen species generation. *Crit Care Med* 38: 871–878, 2010.
57. Hassler R, Scheuner DL, Wang S, Han J, Kodali VK, Li P, Nguyen J, George JS, Davis C, Wu SP, Bai Y, Sartor M, Cavalcoli J, Malhi H, Baudouin G, Zhang Y, Yates JR III, Itkin-Ansari P, Volkmann N, and Kaufman RJ. The IRE1α/XBP1s pathway is essential for the glucose response and protection of β cells. *PLoS Biol* 13: e1002277, 2015.
58. Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, and Holmes KK. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* 174: 1058–1063, 1996.
59. Hecquet CM, Ahmmed GU, Vogel SM, and Malik AB. Role of TRPM2 channel in mediating H_2O_2-induced Ca^{2+} entry and endothelial hyperpermeability. *Circ Res* 102: 347–355, 2008.
60. Helms MN, Jain L, Self JF, and Eaton DC. Redox regulation of epithelial sodium channels examined in alveolar type 1 and 2 cells patch-clamped in lung slice tissue. *J Biol Chem* 283: 22875–22883, 2008.
61. Henriques-Normark B and Normark S. Commensal pathogens, with a focus on *Streptococcus pneumoniae*, and interactions with the human host. *Exp Cell Res* 316: 1408–1414, 2010.
62. Hoskins J, Alborn WE, Jr., Arnold J, Blaszczak LC, Burgett S, DeHoff BS, Estrem ST, Fritz L, Fu DJ, Fuller W, Geringer C, Gilmour R, Glass JS, Khoja H, Kraft AR, Lagace RE, LeBlanc DJ, Lee LN, Lefkowitz EJ, Lu J, Matsushima P, McAhren SM, McHenney M, McLeaster K, Mundy CW, Nicas TI, Norris FH, O'Gara M, Peery RB, Robertson GT, Rockey P, Sun PM, Winkler ME, Yang Y, Young-Bellido M, Zhao G, Zook CA, Baltz RH, Jaskunas SR, Rosteck PR, Jr., Skatrud PL, and Glass JI. Genome of the bacterium *Streptococcus pneumoniae* strain R6. *J Bacteriol* 183: 5709–5717, 2001.
63. Hough RF, Islam MN, Gusarova GA, Jin G, Das S, and Bhattacharya J. Endothelial mitochondria determine rapid barrier failure in chemical lung injury. *JCI Insight* 4: pii: 124329, 2019.
64. Hu L, Zachariae ED, Larsen UG, Vilhardt F, and Petersen SV. The dynamic uptake and release of SOD3 from intracellular stores in macrophages modulates the inflammatory response. *Redox Biol* 26: 101268, 2019.
65. Hua CZ, Howard A, Malley R, and Lu YJ. Effect of nonheme iron-containing ferritin Dpr in the stress response and virulence of pneumococci. *Infect Immun* 82: 3939–3947, 2014
66. Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, and Rossier BC. Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. *Nat Genet* 12: 325–328, 1996.
67. Kooijman EE, Kuzenko SR, Gong D, Best MD, and Folkesson HG. Phosphatidylinositol 4,5-bisphosphate stimulates alveolar epithelial fluid clearance in male and female adult rats. *Am J Physiol Lung Cell Mol Physiol* 301: L804–L811, 2011.
68. Krenn K, Lucas R, Croizé A, Boehme S, Klein KU, Hermann R, Markstaller K, and Ullrich R. Inhaled AP301 for treatment of pulmonary edema in mechanically ventilated patients with acute respiratory distress syndrome: a phase IIa randomized placebo-controlled trial. *Crit Care* 21: 194, 2017.
69. Kube D, Sontich U, Fletcher D, and Davis PB. Proinflammatory cytokine responses to *P. aeruginosa* infection in human airway epithelial cell lines. *Cell Mol Phyiol* 280: L493–L502, 2001.
70. Lee AH, Iwakoshi NN, and Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 23: 7448–7459, 2003.
71. Leung AKC, Wong AHC, and Hon KL. Community-acquired pneumonia in children. *Recent Pat Inflamm Allergy Drug Discov* 12: 136–144, 2018.
72. Li W, Long C, Renjun L, Zhangxue H, Yin H, Wanwei L, Juan M, and Yuan S. Association of SCNN1A single

nucleotide polymorphisms with neonatal respiratory distress syndrome. *Sci Rep* 5: 17317, 2015.
73. Liochev SI and Fridovich I. The effects of superoxide dismutase on H_2O_2 formation. *Free Radic Biol Med* 42: 1465–1469, 2007.
74. Lisher JP, Tsui HT, Ramos-Montañez S, Hentchel KL, Martin JE, Trinidad JC, Winkler ME, and Giedroc DP. Biological and chemical adaptation to endogenous hydrogen peroxide production in *Streptococcus pneumoniae* D39. mSphere 2: pii: e00291-16, 2017.
75. Loose M, Hudel M, Zimmer KP, Garcia E, Hammerschmidt S, Lucas R, Chakraborty T, and Pillich H. Pneumococcal hydrogen peroxide-induced stress signaling regulates inflammatory genes. *J Infect Dis* 211: 306–316, 2015.
76. Lucas R, Echtenacher B, Sablon E, Juillard P, Magez S, Lou J, Donati Y, Bosman F, Van de Voorde A, Fransen L, Männel DN, Grau GE, and de Baetselier P. Generation of a mouse tumor necrosis factor mutant with anti-peritonitis and desensitization activities comparable to those of the wild type but with reduced systemic toxicity. *Infect Immun* 65: 2006–2010, 1997.
77. Lucas R, Sridhar S, Rick FG, Gorshkov B, Umapathy NS, Yang G, Oseghale A, Verin AD, Chakraborty T, Matthay MA, Zemskov EA, White R, Block NL, and Schally AV. Agonist of growth hormone-releasing hormone reduces pneumolysin-induced pulmonary permeability edema. *Proc Natl Acad Sci U S A* 109: 2084–2089, 2012.
78. Lucas R, Verin AD, Black SM, and Catravas JD. Regulators of endothelial and epithelial barrier integrity and function in acute lung injury. *Biochem Pharmacol* 77: 1763–1772, 2009.
79. Lucas R, Yang G, Gorshkov BA, Zemskov EA, Sridhar S, Umapathy NS, Jezierska-Drutel A, Alieva IB, Leustik M, Hossain H, Fischer B, Catravas JD, Verin AD, Pittet JF, Caldwell RB, Mitchell TJ, Cederbaum SD, Fulton DJ, Matthay MA, Caldwell RW, Romero MJ, and Chakraborty T. Protein kinase C-α and arginase I mediate pneumolysin-induced pulmonary endothelial hyperpermeability. *Am J Respir Cell Mol Biol* 47: 445–453, 2012.
80. Luong TT, Kim EH, Bak JP, Nguyen CT, Choi S, Briles DE, Pyo S, and Rhee DK. Ethanol-induced alcohol dehydrogenase E (AdhE) potentiates pneumolysin in *Streptococcus pneumoniae*. *Infect Immun* 83: 108–119, 2015.
81. Lynn H, Sun X, Casanova N, Gonzales-Garay M, Bime C, and Garcia JGN. Genomic and genetic approaches to deciphering acute respiratory distress syndrome risk and mortality. *Antioxid Redox Signal* 31: 1027–1052, 2019.
82. Ma HP. Hydrogen peroxide stimulates the epithelial sodium channel through a phosphatidylinositide 3-kinase-dependent pathway. *J Biol Chem* 286: 32444–32453, 2011.
83. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 53: 401–426, 2013.
84. Malczyk M, Erb A, Veith C, Ghofrani HA, Schermuly RT, Gudermann T, Dietrich A, Weissmann N, and Sydykov A. The role of transient receptor potential channel 6 channels in the pulmonary vasculature. *Front Immunol* 8: 707, 2017.
85. Martín R and Suárez JE. Biosynthesis and degradation of H_2O_2 by vaginal lactobacilli. *Appl Environ Microbiol* 76: 400–405, 2010.
86. Matalon S, Bartoszewski R, and Collawn JF. Role of epithelial sodium channels in the regulation of lung fluid homeostasis. *Am J Physiol Lung Cell Mol Physiol* 309: L1229–L1238, 2015.
87. Matthay MA, Fukuda N, Frank J, Kallet R, Daniel B, and Sakuma T. Alveolar epithelial barrier. Role in lung fluid balance in clinical lung injury. *Clin Chest Med* 21: 477–490, 2000.
88. Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, Herridge M, Randolph AG, and Calfee CS. Acute respiratory distress syndrome. *Nat Rev Dis Primers* 5: 18, 2019.
89. McDevitt CA, Ogunniyi AD, Valkov E, Lawrence MC, Kobe B, McEwan AG, and Paton JC. A molecular mechanism for bacterial susceptibility to zinc. *PLoS Pathog* 7: e1002357, 2011.
90. McLeod JW and Gordon J. Production of hydrogen peroxide by bacteria. *Biochem J* 16: 499–506, 1922.
91. McNeela EA, Burke A, Neill DR, Baxter C, Fernandes VE, Ferreira D, Smeaton S, El-Rachkidy R, McLoughlin RM, Mori A, Moran B, Fitzgerald KA, Tschopp J, Pétrilli V, Andrew PW, Kadioglu A, and Lavelle EC. Pneumolysin activates the NLRP3 inflammasome and promotes proinflammatory cytokines independently of TLR4. *PLoS Pathog* 6: e1001191, 2010.
92. Mishra S and Imlay J. Why do bacteria use so many enzymes to scavenge hydrogen peroxide? *Arch Biochem Biophys* 525: 145–160, 2012.
93. Mitzel DN, Lowry V, Shirali AC, Liu Y, and Stout-Delgado HW. Age-enhanced endoplasmic reticulum stress contributes to increased Atg9A inhibition of STING-mediated IFN-β production during *Streptococcus pneumoniae* infection. *J Immunol* 192: 4273–4283, 2014.
94. Morozov GI, Porat N, Kushnir T, Najmuldeen H, Adawi A, Chalifa-Caspi V, Benisty R, Ohayon A, Liron O, Azriel S, Malka I, Dotan S, Portnoi M, Piotrowski AA, Kafka D, Hajaj B, Fishilevich T, Shagan M, Tal M, Ellis R, Morrison DA, Mitchell AM, Mitchell TJ, Dagan R, Yesilkaya H, and Nebenzahl YM. Flavin reductase contributes to pneumococcal virulence by protecting from oxidative stress and mediating adhesion and elicits protection against pneumococcal challenge. *Sci Rep* 8: 314, 2018.
95. Morty RE and Kuebler WM. TRPV4: an exciting new target to promote alveolocapillary barrier function. *Am J Physiol Lung Cell Mol Physiol* 307: L817–L821, 2014.
96. Muchnik L, Adawi A, Ohayon A, Dotan S, Malka I, Azriel S, Shagan M, Portnoi M, Kafka D, Nahmani H, Porgador A, Gershoni JM, Morrison DA, Mitchell A, Tal M, Ellis R, Dagan R, and Nebenzahl YM. NADH oxidase functions as an adhesin in *Streptococcus pneumoniae* and elicits a protective immune response in mice. *PLoS One* 13: e61128, 2013.
97. Ngocho JS, Magoma B, Olomi GA, Mahande MJ, Msuya SE, de Jonge MI, and Mmbaga BT. Effectiveness of pneumococcal conjugate vaccines against invasive pneumococcal disease among children under five years of age in Africa: a systematic review. *PLoS One* 14: e0212295, 2019.
98. Ong CL, Potter AJ, Trappetti C, Walker MJ, Jennings MP, Paton JC, and McEwan AG. Interplay between manganese and iron in pneumococcal pathogenesis: role of the orphan response regulator RitR. *Infect Immun* 81: 421–429, 2013.
99. Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, and Mori M. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest* 109: 525–532, 2002.

100. Paterson GK, Blue CE, and Mitchell TJ. An operon in *Streptococcus pneumoniae* containing a putative alkylhydroperoxidase D homologue contributes to virulence and the response to oxidative stress. *Microb Pathog* 40: 152–160, 2006.
101. Paudel S, Baral P, Ghimire L, Bergeron S, Jin L, DeCorte JA, Le JT, Cai S, and Jeyaseelan S. CXCL1 regulates neutrophil homeostasis in pneumonia-derived sepsis caused by *Streptococcus pneumoniae* serotype 3. *Blood* 133: 1335–1345, 2019.
102. Pearse DB, Shimoda LA, Verin AD, Bogatcheva N, Moon C, Ronnett GV, Welsh LE, and Becker PM. Effect of cGMP on lung microvascular endothelial barrier dysfunction following hydrogen peroxide. *Endothelium* 10: 309–317, 2003.
103. Pericone CD, Overweg K, Hermans PW, and Weiser JN. Inhibitory and bactericidal effects of *Streptococcus pneumoniae* on other inhabitants of the upper respiratory tract. *Infect Immun* 68: 3990–3997, 2000.
104. Pericone CD, Park S, Imlay JA, and Weiser JN. Factors contributing to hydrogen peroxide resistance in *Streptococcus pneumoniae* include pyruvate oxidase (SpxB) and avoidance of the toxic effects of the fenton reaction. *J Bacteriol* 185: 6815–6825, 2003.
105. Petry A, Zhang Z, Trautz B, Rieß F, and Görlach A. Cross talk between p22phox and ATF4 in the endothelial unfolded protein response. *Antioxid Redox Signal* 30: 40–55, 2019.
106. Pillich H, Loose M, Zimmer K, and Chakraborty T. Activation of the unfolded protein response by Listeria monocytogenes. *Cell Microbiol* 14: 949–964, 2012.
107. Piñas GE, Reinoso-Vizcaino NM, Yandar Barahona NY, Cortes PR, Duran R, Badapanda C, Rathore A, Bichara DR, Cian MB, Olivero NB, Perez DR, and Echenique J. Crosstalk between the serine/threonine kinase StkP and the response regulator ComE controls the stress response and intracellular survival of *Streptococcus pneumoniae*. *PLoS Pathog* 14: e1007118, 2018.
108. Pochynyuk O, Tong Q, Staruschenko A, Ma HP, and Stockand JD. Regulation of the epithelial Na+ channel (ENaC) by phosphatidylinositides. *Am J Physiol Renal Physiol* 290: F949–F957, 2006.
109. Rai P, Parrish M, Tay IJ, Li N, Ackerman S, He F, Kwang J, Chow VT, and Engelward BP. *Streptococcus pneumoniae* secretes hydrogen peroxide leading to DNA damage and apoptosis in lung cells. *Proc Natl Acad Sci U S A* 112: E3421–E3430, 2015.
110. Raju S, Painter R, Bagby G, Nelson S, and Wang G. Response of differentiated human airway epithelia to alcohol exposure and *Klebsiella pneumoniae* challenge. *Med Sci* 1: 2–19, 2013.
111. Rubins JB, Duane PG, Clawson D, Charboneau D, Young J, and Niewoehner DE. Toxicity of pneumolysin to pulmonary alveolar epithelial cells. *Infect Immun* 61: 1352–1358, 1993.
112. Rzymski T, Petry A, Kračun D, Rieß F, Pike L, Harris AL, and Görlach A. The unfolded protein response controls induction and activation of ADAM17/TACE by severe hypoxia and ER stress. *Oncogene* 31: 3621–3634, 2012.
113. Sakaguchi R and Mori Y. Transient receptor potential (TRP) channels: biosensors for redox environmental stimuli and cellular status. *Free Radic Biol Med* 19: 30701–30704, 2019.
114. Schmeck B, N'Guessan PD, Ollomang M, Lorenz J, Zahlten J, Opitz B, Flieger A, Suttorp N, and Hippenstiel S. *Legionella pneumophila* induced NF-kappaB- and MAPK-dependent cytokine release by lung epithelial cells. *Eur Respir J* 29: 25–33, 2006.
115. Seeger W, Hansen T, Rössig R, Schmehl T, Schütte H, Krämer HJ, Walmrath D, Weissmann N, Grimminger F, and Suttorp N. Hydrogen peroxide-induced increase in lung endothelial and epithelial permeability-effect of adenylate cyclase stimulation and phosphodiesterase inhibition. *Microvasc Res* 50: 1–17, 1995.
116. Shlyonsky V, Boom A, and Mies F. Hydrogen peroxide and sodium transport in the lung and kidney. *Biomed Res Int* 2016: 9512807, 2016.
117. Simmons S, Erfinanda L, Bartz C, and Kuebler WM. Novel mechanisms regulating endothelial barrier function in the pulmonary microcirculation. *J Physiol* 597: 997–1021, 2019.
118. Spellerberg B, Cundell DR, Sandros J, Pearce BJ, Idanpaan-Heikkila I, Rosenow C, and Masure HR. Pyruvate oxidase, as a determinant of virulence in *Streptococcus pneumoniae*. Mol Microbiol 19: 803–813, 1996.
119. Subramanian K, Henriques-Normark B, and Normark S. Emerging concepts in the pathogenesis of the *Streptococcus pneumoniae*: from nasopharyngeal colonizer to intracellular pathogen. *Cell Microbiol* 11: e13077, 2019
120. Suresh K, Servinsky L, Reyes J, Undem C, Zaldumbide J, Rentsendorj O, Modekurty S, Dodd-O JM, Scott A, Pearse DB, and Shimoda LA. CD36 mediates H_2O_2-induced calcium influx in lung microvascular endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 312: L143–L153, 2017.
121. Syk A, Norman M, Fernebro J, Gallotta M, Farmand S, Sandgren A, Normark S, and Henriques-Normark B. Emergence of hypervirulent mutants resistant to early clearance during systemic serotype 1 pneumococcal infection in mice and humans. *J Infect Dis* 210: 4–13, 2014.
122. Taniai H, Iida K, Seki M, Saito M, Shiota S, Nakayama H, and Yoshida S. Concerted action of lactate oxidase and pyruvate oxidase in aerobic growth of *Streptococcus pneumoniae*: role of lactate as an energy source. *J Bacteriol* 190: 3572–3579, 2008.
123. Tettelin H, Nelson KE, Paulsen IT, Eisen JA, Read TD, Peterson S, Heidelberg J, DeBoy RT, Haft DH, Dodson RJ, Durkin AS, Gwinn M, Kolonay JF, Nelson WC, Peterson JD, Umayam LA, White O, Salzberg SL, Lewis MR, Radune D, Holtzapple E, Khouri H, Wolf AM, Utterback TR, Hansen CL, McDonald LA, Feldblyum TV, Angiuoli S, Dickinson T, Hickey EK, Holt IE, Loftus BJ, Yang F, Smith H, Venter J, Dougherty BA, Morrison DA, Hollingshead SK, and Fraser CM. Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. *Science* 293: 498–506, 2001.
124. Tseng HJ, McEwan AG, Paton JC and Jennings MP. Virulence of *Streptococcus pneumoniae*: PsaA mutants are hypersensitive to oxidative stress. *Infect Immun* 70: 1635–1639, 2002.
125. Turner AG, Ong CL, Gillen CM, Davies MR, West NP, McEwan AG, and Walker MJ. Manganese homeostasis in group A *Streptococcus* is critical for resistance to oxidative stress and virulence. *mBio* 6: e00278-15, 2015.
126. Vadász I and Lucas R. Editorial: cytokine-ion channel interactions in pulmonary inflammation. *Front Immunol* 9: 2598, 2018.
127. Vadász I and Sznajder JI. Gas exchange disturbances regulate alveolar fluid clearance during acute lung injury. *Front Immunol* 8: 757, 2017.

128. Walker JA, Allen RL, Falmagne P, Johnson MK, and Boulnois GJ. Molecular cloning, characterization, and complete nucleotide sequence of the gene for pneumolysin, the sulfhydryl-activated toxin of *Streptococcus pneumoniae*. *Infect Immun* 55: 1184–1189, 1987.
129. Walter JM, Ren Z, Yacoub T, Reyfman PA, Shah RD, Abdala-Valencia H, Nam K, Morgan VK, Anekalla KR, Joshi N, McQuattie-Pimentel AC, Chen CI, Chi M, Han S, Gonzalez-Gonzalez FJ, Soberanes S, Aillon RP, Watanabe S, Williams KJN, Lu Z, Paonessa J, Hountras P, Breganio M, Borkowski N, Donnelly HK, Allen JP, Amaral LA, Bharat A, Misharin AV, Bagheri N, Hauser AR, Budinger GRS, and Wunderink RG. Multidimensional assessment of the host response in mechanically ventilated patients with suspected pneumonia. *Am J Respir Crit Care Med* 199: 1225–1237, 2019.
130. Wandall-Frostholm C, Dalsgaard T, Bajoriunas V, Olivan-Viguera A, Sadda V, Beck L, Mogensen S, Stankevicius E, Simonsen U, and Kohler R. Genetic deficit of KCa 3.1 channels protects against pulmonary circulatory collapse induced by TRPV4 channel activation. *Br J Pharmacol* 172, 4493–4505, 2015.
131. Wang HC, Zentner MD, Deng HT, Kim KJ, Wu R, Yang PC, and Ann DK. Oxidative stress disrupts glucocorticoid hormone-dependent transcription of the amiloride-sensitive epithelial sodium channel alpha-subunit in lung epithelial cells through ERK-dependent and thioredoxin-sensitive pathways. *J Biol Chem* 275: 8600–8609, 2000.
132. Ware LB and Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med.* 163: 1376–1383, 2001.
133. Weibel ER. Lung morphometry: the link between structure and function. *Cell Tissue Res* 367: 413–426, 2017.
134. Weissmann N, Sydykov A, Kalwa H, Storch U, Fuchs B, Mederos Y, Schnitzler M, Brandes RP, Grimminger F, Meissner M, Freichel M, Offermanns S, Veit F, Pak O, Krause KH, Schermuly RT, Brewer AC, Schmidt HH, Seeger W, Shah AM, Gudermann T, Ghofrani HA, and Dietrich A. Activation of TRPC6 channels is essential for lung ischaemia-reperfusion induced oedema in mice. *Nat Commun* 3: 649, 2012.
135. West JB. Role of the fragility of the pulmonary blood-gas barrier in the evolution of the pulmonary circulation. *Am J Physiol Regul Integr Comp Physiol* 304: R171–R176, 2013.
136. Winterbourn CC, Kettle AJ, and Hampton MB. Reactive oxygen species and neutrophil function. *Annu Rev Biochem* 85: 765–792, 2016.
137. Witzenrath M, Gutbier B, Hocke AC, Schmeck B, Hippenstiel S, Berger K, Mitchell TJ, de los Toyos JR, Rosseau S, Suttorp N, and Schütte H. Role of pneumolysin for the development of acute lung injury in pneumococcal pneumonia. *Crit Care Med* 34: 1947–1954, 2006.
138. Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J, Song B, Yau GD, and Kaufman RJ. ATF6α optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. *Dev Cell* 13: 351–364, 2007.
139. Wu X, Gordon O, Jiang W, Antezana BS, Angulo-Zamudio UA, Del Rio C, Moller A, Brissac T, Tierney ARP, Warncke K, Orihuela CJ, Read TD, and Vidal JE. Interaction between *Streptococcus pneumoniae* and *Staphylococcus aureus* generates $^{\bullet}$OH radicals that rapidly kill *Staphylococcus aureus* strains. *J Bacteriol* 201: e00474-19, 2019.
140. Wuerges J, Lee JW, Yim YI, Yim HS, Kang SO, and Djinovic Carugo K. Crystal structure of nickel-containing superoxide dismutase reveals another type of active site. *Proc Natl Acad Sci U S A* 101: 8569–8574, 2004.
141. Xu H and Chu S. ENaC alpha-subunit variants are expressed in lung epithelial cells and are suppressed by oxidative stress. *Am J Physiol Lung Cell Mol Physiol* 293: L1454–L1462, 2007.
142. Yesilkaya H, Andisi VF, Andrew PW, and Bijlsma JJ. *Streptococcus pneumoniae* and reactive oxygen species: an unusual approach to living with radicals. *Trends Microbiol* 21: 187–195, 2013.
143. Yesilkaya H, Kadioglu A, Gingles N, Alexander JE, Mitchell TJ, and Andrew PW. Role of manganese-containing superoxide dismutase in oxidative stress and virulence of *Streptococcus pneumoniae*. *Infect Immun* 68: 2819–2826, 2000.
144. Yonezawa R, Yamamoto S, Takenaka M, Kage Y, Negoro T, Toda T, Ohbayashi M, Numata T, Nakano Y, Yamamoto T, Mori Y, Ishii M, and Shimizu S. TRPM2 channels in alveolar epithelial cells mediate bleomycin-induced lung inflammation. *Free Radic Biol Med* 90: 101–113, 2016.
145. Yoshida H, Matsui T, Yamamoto A, Okada T, and Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107: 881–891, 2001.
146. Yu J, Bryant AP, Marra A, Lonetto MA, Ingraham KA, Chalker AF, Holmes DJ, Holden D, Rosenberg M, and McDevitt D. Characterization of the *Streptococcus pneumoniae* NADH oxidase that is required for infection. *Microbiol* 147: 431–438, 2001.
147. Yue G, Malik B, Yue G and Eaton DC. Phosphatidylinositol 4,5-bisphosphate (PIP2) stimulates epithelial sodium channel activity in A6 cells. *J Biol Chem* 277: 11965–11969, 2002.
148. Zhang K and Kaufman RJ. Signaling the unfolded protein response from the endoplasmic reticulum. *J Biol Chem* 279: 25935–25938, 2004.

Address correspondence to:
Prof. Trinad Chakraborty
Institute for Medical Microbiology
Justus-Liebig University
Giessen, Hessen 35392
Germany

E-mail: trinad.chakraborty@mikrobio.med.uni-giessen.de

Prof. Rudolf Lucas
Department of Medicine
Medical College of Georgia at Augusta University
Augusta, GA 30912
USA

E-mail: rlucas@augusta.edu

Date of first submission to ARS Central, November 28, 2019; date of final revised submission, March 17, 2020; date of acceptance, April 3, 2020.

Abbreviations Used

Ac-P = acetyl phosphate
AdhE = aldehyde-alcohol dehydrogenase E
ADP = adenosine diphosphate
AFC = alveolar fluid clearance
AhpD = alkyl hydroperoxide reductase
ARDS = acute respiratory distress syndrome
ATF4 = activating transcription factor 4
ATF6 = activating transcription factor 6
ATP = adenosine triphosphate
BiP = immunglobulin heavy-chain-binding protein
Ca^{2+} = calcium
cAMP = cyclic adenosine monophosphate
CAP = community-acquired pneumonia
CC = clonal complex
CcdA = cytochrome *c*-type biogenesis protein homologue
CD36 = cluster of differentiation 36
CDC = cholesterol-dependent cytolysin
cGMP = cyclic guanosine monophosphate
CiaRH = *ciaH* = histidine kinase gene
ClpP = ATP-dependent Clp protease proteolytic subunit
CO_2 = carbon dioxide
CodY = GTP-sensing transcriptional pleiotropic repressor
ComE = response regulator ComE
CTM = CcdA, TlpA, MsrAB
Dpr = Dps-like peroxide resistance
Dps = DNA-binding protein from starved cells
DUOX1/2 = dual oxidase 1/2
eiF2α = eukaryotic translation initiation factor 2α
ENaC = epithelial sodium channel
ER = endoplasmic reticulum
ERAD = endoplasmic-reticulum-associated protein degradation
ERK = extracellular signal-regulated kinases
FabF = 3-oxoacyl-[acyl-carrier-protein] synthase 2
Fyn = proto-oncogene tyrosine-protein kinase Fyn
H_2O_2 = hydrogen peroxide
HO-1 = heme oxygenase 1
HtrA = high-temperature requirement A
IκBα = nuclear factor of kappa light polypeptide gene enhancer in B cell inhibitor, alpha
IFN-I = type I interferon
IL = interleukin
IRE1 = inositol-requiring enzyme 1
JNK = c-Jun N-terminal kinase
LctO = lactate oxidase
LPS = lipopolysaccharide
MAPK = mitogen-associated protein kinase
MerR = mercury-sensing regulatory protein
MLCK = myosin light-chain kinase
MPO = myeloperoxidase
mSOFA = modified sequential organ failure assessment
MsrAB = methionine sulfoxide reductase A/B protein
mtDNA = mitochondrial DNA
mtROS = mitochondrial ROS
Na^+–K^+ pump = sodium–potassium pump
ND = not determined
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
Nox-2 = NADPH oxidase 2
Nrf-2 = nuclear factor erythroid 2-related factor 2
O_2 = oxygen
OxyR = hydrogen peroxide-inducible genes activator
PERK = protein kinase R (PKR)-like ER kinase
PerR = ferric uptake regulator
PI3-kinase = phosphoinositide 3-kinase
PIP_2 = phosphatidylinositol-4,5-bisphosphate
PIP_3 = phosphatidylinositol-3,4,5-trisphosphate
Ply = pneumolysin
PsaA = pneumococcal surface adhesin A
RelA = nuclear factor NF-kappa-B p65 subunit
RIDD = regulated Ire1-dependent decay
RitR = orphan response regulator
ROS = reactive oxygen species
S1P = site-1-protease
S2P = site-2-protease
siRNA = silencing RNA
SOD = superoxide dismutase
Spn = *Streptococcus pneumoniae*
SpxB = pyruvate oxidase
STING = stimulator of interferon genes
StkP = serine/threonine kinase
TCS04 = two-component system 4
TlpA = thioredoxin-like protein family
TNF = tumor necrosis factor
TpxD = thiol peroxidase
TRP = transient receptor potential
TRPC6 = transient receptor potential channel 6
TRPM2 = transient receptor potential melastatin 2
TRPV2 = transient receptor potential vanilloid
UPR = unfolded protein response
VILI = ventilator-induced lung injury
XBP1 = X-box-binding protein 1