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Reactive Nitrogen and Oxygen Intermediates and Bacterial Defenses: Unusual Adaptations in *Mycobacterium tuberculosis*

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ABSTRACT

The production of reactive oxygen and reactive nitrogen intermediates is an important host defense mechanism mediated in response to infection by bacterial pathogens. Not surprisingly, intracellular pathogens have evolved numerous defense strategies to protect themselves against the damaging effects of these agents. In enteric bacteria, exposure to oxidative or nitrosative stress induces expression of numerous pathways that allow the bacterium to resist the toxic effects of these compounds during growth in the host. In contrast, members of pathogenic mycobacterial species, including the frank human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, are dysfunctional in aspects of the oxidative and nitrosative stress response, yet they remain able to establish and maintain productive acute and persistent infections in the host. This article reviews the current knowledge regarding reactive oxygen and nitrogen intermediates, and compares the adaptative mechanisms utilized by enteric organisms and mycobacterial species to resist the bactericidal and bacteriostatic effects resulting from exposure to these compounds. Antioxid. Redox Signal. 4, 141–159.

HOST CELL GENERATION OF REACTIVE OXYGEN AND NITROGEN INTERMEDIATES

Specialized mammalian cells of the immune system utilize a variety of mechanisms to control infection by bacterial pathogens. A subset of these mechanisms includes the production of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). These agents, generated under physiological conditions, are capable of damaging DNA bases and lipids, and disrupting the activity of important cellular proteins containing Fe-S clusters, transition metals, hemes, thiols, sulfhydryl, or tyrosyl groups (84, 85, 115). Damage mediated by these compounds leads to a general inhibition in cellular metabolism by altering processes associated with proton-dependent active transport, oxygen utilization, and oxidative phosphorylation (37, 84). Although most eukaryotic cells generate low levels of ROI and RNI, macrophages have evolved specialized systems that allow them to be potent generators of both as needed, due in part to their ability to induce expression of nitric oxide synthase 2 (iNOS or NOS2) and assemble components of the NADPH oxidase (Phox).

There are three categories of oxidants generated by the Phox oxidase and iNOS (Fig. 1). First, NADPH oxidase generates ROI through

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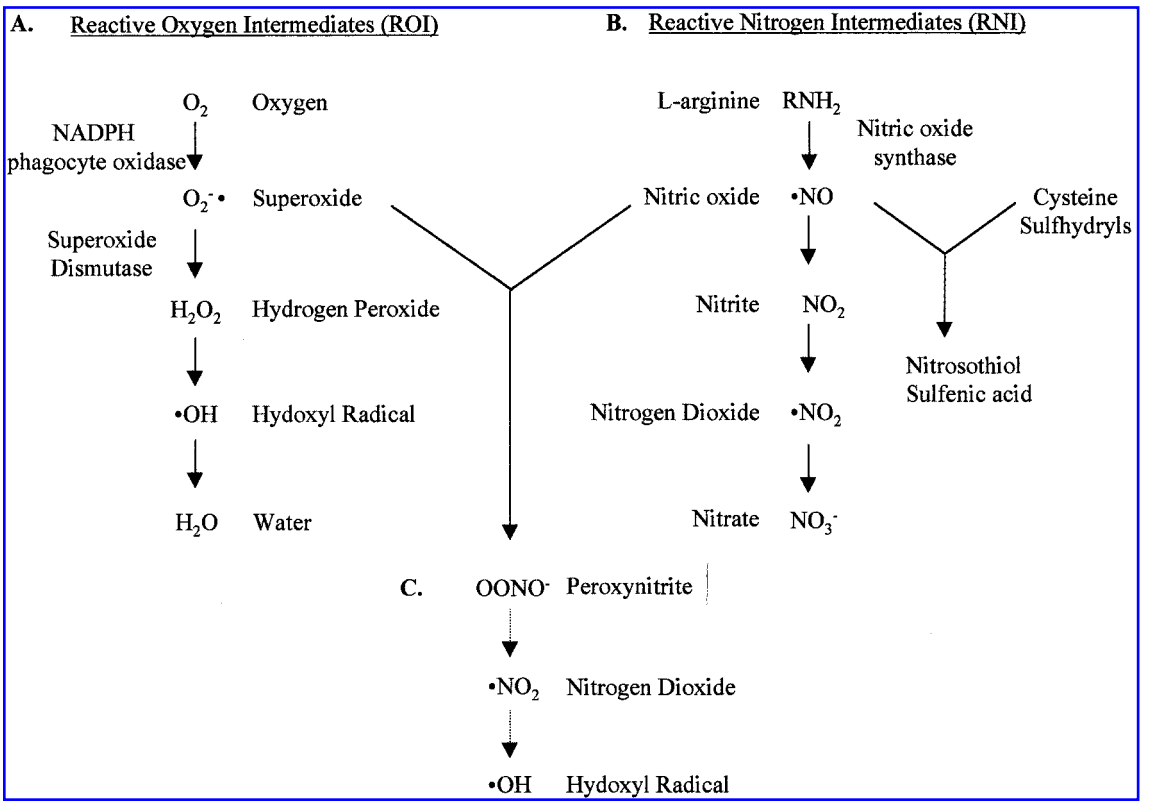

FIG. 1. Pathways of ROI and RNI generation. (**A**) ROI including superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxy radical (·OH) are generated by NADPH phagocyte oxidase (Phox) in the presence of iron and upon superoxide dismutation. (**B**) The inducible nitric oxide synthase (iNOS) generates RNI including nitric oxide (·NO) and its various oxidative products including nitrite (NO_2^-), nitrogen dioxide ($\cdot NO_2$) and nitrate (NO_3^-) in the presence of substrate L-arginine. The reaction of nitric oxide with cysteine sulfhydryls results in the generation of nitrosothiols and sulfenic acids. (**C**) The interaction of $O_2^{-\cdot}$ generated by Phox and ·NO generated by iNOS combine to form the toxic intermediate peroxynitrite ($OONO^-$) and its various decomposition products.

the reduction of molecular oxygen. The primary products generated from this reaction are superoxide ($O_2^{-\cdot}$) and its various dismutation and decomposition products, including hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), an especially strong oxidant in biological systems (108). Second, iNOS uses L-arginine as a substrate to generate nitric oxide (·NO). A stepwise oxidation of nitric oxide with oxygen to nitrate (NO_3^-) results in production of various intermediates including nitrite (NO_2^-) and nitrogen dioxide ($\cdot NO_2$). Following interaction of nitric oxide with cysteine residues, sulfhydryls and glutathione or its equivalents can be converted into nitrosothiols or sulfenic acid (85). A third category of reactive intermediates occurs when the products of NADPH oxidase and iNOS interact to form highly potent antibacterial compounds. For example, peroxynitrite ($OONO^-$) is generated following the reaction of superoxide with nitric oxide, and is one of the most potent natural oxidants in biological systems (93). Apart from NADPH oxidase and nitric oxide synthase (NOS), other leukocyte enzymes contribute to the generation of ROI. Production of HOCl and HOBr is mediated by eukaryotic myeloperoxidases in a reaction dependent on the peroxidation of halide ions in the presence of H_2O_2 (42, 67). HOCl and HOBr are highly destructive and also act on a variety of cellular components, including enzymes (5) and DNA (32, 59). HOCl generates hydroxyl radicals in the presence of Fe^{2+} or Fe^{3+} (60) or superoxide anions (12), and oxygen singlet radicals upon acidification (65, 66) or interaction with H_2O_2 (55, 58).

Although the generation of ROI and RNI is beneficial to the host in defense against bacterial infection (37), unregulated production of these agents can also lead to extensive host cell damage (85). RNI have been shown to in-

hibit the activity of G proteins, activate or inhibit protein kinases, caspases, metalloproteases, and transcription factors, and promote apoptosis of host cells (85). Expression of ROI and RNI has also been implicated in cellular and tissue dysfunction in sepsis (47).

Murine phagocytic cells have been the main model system used by investigators to study effects of ROI and RNI generation in the context of host–pathogen interactions. The utility of murine macrophages is attributed in part to their ability to induce expression of significant levels of Phox and iNOS following stimulation with various cytokines, including interleukin-1, interferon-γ, and tumor necrosis factor-α, and/or treatment with bacterial products such as lipopolysaccharide or lipoteichoic acid *in vitro* (37). Adding to their usefulness has been the isolation of murine macrophage cell lines defective in the ability to generate ROI (46) and transgenic mice genetically engineered to carry *phox*$^{-/-}$ and/or *nos2*$^{-/-}$ mutations (61, 87, 98, 116). The extent of the relevance of these animal systems for modeling aspects of host–pathogen interactions in humans is presently unclear. For example, mice carrying *phox* mutations are resistant to spontaneous infection by *Salmonella typhimurium* and other pathogenic organisms (116), although humans that carry genetic deficiencies in components of the NADPH oxidase suffer from chronic granulomatous disease (CGD), a condition characterized by extreme susceptibilty to bacterial and fungal infections (103). Adding to this controversy is the ongoing debate regarding iNOS expression in human macrophages. Whereas tissue macrophages from rodents readily induce expression of iNOS following stimulation with microbial products or cytokines (115), administration of the same stimuli to mononuclear phagocytes cultured from the blood of healthy humans does not consistently induce iNOS expression (115). However, iNOS expression at high levels can be consistently observed in human tissue macrophages from inflamed or infected sites (133).

BACTERIAL DEFENSE MECHANISMS

Bacterial pathogens have evolved a variety of mechanisms to counteract host cell production of ROI and RNI. A subset of these processes involve the expression of genes whose products interfere with synthesis of reactive intermediates, allow the direct catabolization of intermediary products, or participate in the repair of ROI- and RNI-generated DNA damage (115). Yet another more recent mechanism utilized by bacterial pathogens to counteract ROI and RNI production is exclusion of parts of the NADPH oxidase machinery from the phagosome (129). The responses of microorganisms to ROI and RNI have been best studied in enteric bacteria such as *Escherichia coli* and *Salmonella typhimurium*, which currently serve as the archetypical model systems for the study of oxidative and nitrosative stress responses. These bacterial species have proven useful in the elucidation of ROI and RNI resistance mechanisms, as they can be genetically manipulated, are important intracellular pathogens of humans, and have relevant *in vitro* and animal model systems of infection.

THE ENTERIC PARADIGM: THE OXIDATIVE STRESS RESPONSE

The oxidative stress response in *S. typhimurium* and *E. coli* is primarily mediated by the regulated expression of two global transcription factors, OxyR and SoxR (Table 1) (122). OxyR regulates the expression of genes in response to peroxide stress, whereas the *soxRS* regulon regulates the expression of genes in response to superoxide. These transcriptional factors were originally identified in screens for *E. coli* mutants displaying increased resistance to ROI (18, 50). Together, these determinants regulate a subset of the genes collectively induced following exposure of *E. coli* and *S. typhimurium* to various generators of oxidative stress (49, 81, 126, 131). Although similar in that they both modulate expression of downstream gene targets, OxyR and SoxR utilize different mechanisms to mediate their adaptive redox response. Activation of OxyR occurs by a conformational change that results from the direct oxidation of two thiol groups and formation of an intramolecular disulfide bond (17). In contrast, activation of SoxR into its transcriptionally

TABLE 1. GENES REGULATED BY OXYR AND SOXRS IN ENTERIC ORGANISMS*

Regulator	*Gene*	*Function*	*Activity*	*Mechanism*
OxyR				
	katG	Catalase-peroxidase	Detoxification of peroxides	Activation
	ahpCF	Alkylhydroperoxidase reductase	Detoxification of peroxides	Activation
	gorA	Gluathione reductase	Maintain cellular thiol-disulfide balance	Activation
	grxA	Glutaredoxin 1	Maintain cellular thiol-disulfide balance	Activation
	trxC	Thioredoxin 2	Maintain cellular thiol-disulfide balance	Activation
	dps	ssDNA binding protein	Sequester DNA against damage and mutation	Activation
	fur	Ferric uptake regulator	Prevent damage by ·OH formation	Activation
	oxyS	Small RNA	Protect against mutagenesis; transcriptional regulator	Activation
	oxyR		Autogenous regulation	Repression
	agn43	A phase-variable antigen of the outer membrane	Unknown	Repression
	fhuF	Ferric reductase	Minimize ferric iron uptake and prevent Fenton reaction	Repression
SoxRS				
	sodA	Mn-containing superoxide dismutase	Dismutation of superoxide to water	Activation
	zwf	Glucose-6-phosphate dehydrogenase	Increase reducing power of cell	Activation
	nfo	Endonuclease IV	Repair DNA damage	Activation
	fumC	Fumurase C	Isozyme form resistant to superoxide	Activation
	acnA	Aconitase A	Isozyme form resistant to superoxide	Activation
	tolC	Outer membrane protein	Exclusion of $O_2^{-\cdot}$ generating compounds	Activation
	fur	Ferric uptake regulator	Minimize formation of ·OH via Fenton reaction	Activation
	micF	RNA regulator of *ompF*	Exclusion of $O_2^{-\cdot}$ generating compounds	Activation
	acrAB	Multidrug efflux pump	Exclusion of $O_2^{-\cdot}$ generating compounds	Activation
	nfsA	Nitroreductase A	Diminish $O_2^{-\cdot}$ production by redox cycling	Activation
	fpr	Ferredoxin/flavodoxin reductase	Maintain reduced state of Fe-S clusters	Activation
	fldA	Flavodoxin	Maintain reduced state of Fe-S clusters	Activation
	fldB	Flavodoxin	Maintain reduced state of Fe-S clusters	Activation
	ribA	GTP hydrolase	Unknown	Activation

*Reviewed in 122.

active form requires direct oxidation of a [2Fe-2S] cluster (45). The ability to modulate antioxidant activity by differential redox sensing mechanisms likely enhances the ability of these organisms to fine-tune their adaptation.

The activation of *oxyR* and *soxRS* in enteric bacteria induces the expression of a number of genes that participate in resistance to oxidative stress (Table 1). OxyR positively regulates the catalase-peroxidase *katG*, the glutathione reductase *gorA*, the alkylhydroperoxidase reductase *ahpCF*, a nonspecific DNA binding protein *dps*, the ferric uptake regulator *fur*, and several other genes including *grxA*, *trxC*, *oxyS*, *oxyR*, *agn43*, and *fhuF* (122). Activation of *soxRS* induces the expression of a different subset of genes including *sodA*, a Mn-containing superoxide dismutase, *zwf* encoding glucose-6-phosphate dehydrogenase, *nfo* encoding the DNA repair endonuclease IV, as well as several other genes including *fumC*, *acnA*, *tolC*, *fur*, *micF*, *acrAB*, *nfsA*, *fpr*, *fldA*, *fldB*, and *ribA* (122). The importance of *oxyR* and *soxRS*, and the genes regulated by these determinants, for resistance to ROI *in vitro* is well established and will not be discussed here (121, 122). In contrast, the role for these genes in growth of enteric bacteria *in vivo* is not well defined and is somewhat controversial. For example, although *ahpC* is induced in *S. typhimurium* during growth in murine macrophages (43), and *ahpC* (124) and *katG* (63) are both antigens found during the course of infection, mutations in these two antioxidant genes do not attenuate *S. typhimurium* for virulence in the murine model of salmonellosis (95, 124). Similarly, *S. ty-*

phimurium strains carrying mutations in *oxyR* or the *soxRS* regulon, or strains that overexpress functional *oxyR,* are not more sensitive to macrophage or neutrophil killing *in vitro* (38, 95) and are not attenuated for virulence *in vivo* (95, 124). However, expression of glucose-6-phosphate dehydrogenase (*zwf*), a *soxR*-regulated enzyme from the pentose phosphate cycle, is required for virulence of *S. typhimurium in vivo* (71). Taken together, these results provide conflicting conclusions regarding the role of *oxyR*- and *soxRS*-induced resistance mechanisms in host–pathogen interactions involving enteric organisms. As *oxyR* and *soxRS* normally function to increase transcription of target genes under conditions of oxidative stress, basal level expression of these and other *oxyR*- and *soxR*-dependent genes may be sufficient to provide adequate enzymatic activity to sustain viability under such conditions. Alternatively, it is important to keep in mind that enteric organisms switch between fermentation and oxidative metabolism and that this change, without any external oxidative insult, represents a challenge that these bacteria must deal with. Thus, some of these systems may be primarily designed to facilitate transition from fermentation to oxidative metabolism, but secondarily have been recruited in the defense against oxidants produced by mammalian defense cells.

Other genes involved in resistance to oxidative stress, but in a manner independent of *oxyR* and *soxRS,* also contribute to ROI resistance in enteric organisms. These include genes such as the catalase gene *katE,* the DNA polymerase I *polA,* the DNA repair enzymes exonuclease III *xthA,* the DNA recombination enzymes *recA* and *recBCD,* and superoxide dismutases such as *sodC* and *sodB* (122). Mutations in many of these genes also result in increased sensitivity to *in vitro*-generated oxidants (122) and attenuate virulence during infection *in vivo* (11, 24, 39).

THE ENTERIC NITROSATIVE STRESS RESPONSE

Although a great deal of information is known about bacterial mechanisms mediating resistance to ROI in enteric bacteria, relatively few genes have been identified that participate in resistance to RNI. The *hmp* gene encodes a flavohemoglobin that has been shown to be a nitric oxide detoxifying enzyme in *E. coli* (54). In addition, *Salmonella* strains unable to synthesize glutathione are hypersusceptible to inhibition by *S*-nitrosothiols, peroxynitrite, and nitric oxide itself (22), presumably due to an inability to scavenge RNI. Similarly, *Salmonella* mutants unable to synthesize homocysteine, another low-molecular-weight thiol, are hypersusceptible to *S*-nitrosoglutathione *in vitro* (23) and are attenuated during growth in macrophages *in vitro* and during growth in mice (23). Interestingly, analysis of the enteric response to RNI suggests that enteric organisms may utilize parts of the oxidative stress response to mediate resistance against RNI. For example, the *E. coli oxyR* regulon is induced by and confers resistance to *S*-nitrosothiols *in vitro* (53). In addition, *S. typhimurium* AhpC has recently been shown to possess peroxynitritase activity (9). Other genes such as glucose-6-phosphate dehydrogenase, a gene regulated by *soxR,* confers resistance against nitric oxide donor compounds to *S. typhimurium in vitro* (71). In addition, *oxyR*- and *soxR*-independent genes induced in response to oxidative stress may also help protect enteric organisms against RNI including *sodC,* which may act to limit peroxynitrite formation in the periplasm (24). Thus, although it remains unclear whether a dedicated antinitrosative regulon exists in enteric bacteria, resistance of these organisms to RNI is likely to be a result of the coordinated expression of genes from multiple pathways, enhancing the organism's ability to survive the short-lived bactericidal phase generated by the respiratory burst oxidase and the longer-lived bacteriostatic phase supported by inducible expression of NOS.

ROLE OF ROI AND RNI IN CONTROL OF PATHOGENIC MYCOBACTERIA

Most virulent mycobacterial species infect and reside in macrophages or other phagocytic cells of the host. Thus, the necessity for

these species to resist the bactericidal and bacteriostatic effects of ROI and RNI becomes readily apparent when one considers the environment in which they are located. As an added challenge, mycobacteria must be able to resist extensive periods of exposure to ROI and RNI, as they frequently establish long-term, latent infections in their host. *Mycobacterium tuberculosis,* the causative agent of human tuberculosis and the most studied of the pathogenic mycobacteria, infects lung macrophages of susceptible hosts. Within these normally bactericidal cells, *M. tuberculosis* is capable of surviving and maintaining a persistent infection that frequently lasts the lifetime of the individual.

INFECTION WITH *M. tuberculosis*: STAGES OF DISEASE AND INTERACTIONS WITH HOST MACROPHAGES

Approximately 1.7 billion people are currently infected with *M. tuberculosis* (35), a staggering statistic considering that public health officials had declared tuberculosis no longer a significant health concern 20 years ago (8). Tuberculosis remains the leading cause of death in the world from a single infectious agent (8) and results in nearly 3 million deaths a year (101). Although deaths from tuberculosis in the United States and other industrialized countries have continued to decline steadily in recent years due to the administration of effective therapeutic drug regimens and the installation of worldwide direct observation therapy support, the emergence of multiple drug-resistant isolates, the lack of novel therapeutics effective against tubercle bacilli in the persistent stages of infection, and the lack of a universally effective vaccine continue to threaten the ability of health care workers to treat tuberculosis effectively and prevent its further expansion in the human population.

M. tuberculosis is typically acquired by the inhalation of droplet nuclei containing a small number of tubercle bacilli (Figure 2). Following inhalation, *M. tuberculosis* infects the resident alveolar macrophages of the lung, which, in individuals contracting disease or showing

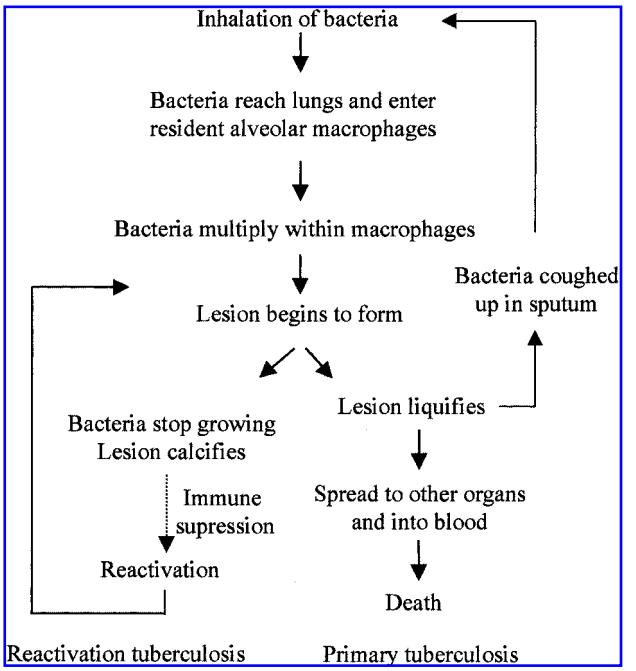

FIG. 2. Life cycle of *Mycobacterium tuberculosis*. *M. tuberculosis* acquired by inhalation resides and multiplies in lung macrophages. Bacteria replicate during the innate stages of immunity and reach high numbers in the macrophages of immunodeficient hosts where they cause active disease. If untreated, organisms spread to other parts of the body and can cause death of the host. Bacteria infecting immunocompetent hosts are controlled by cell-mediated immunity and either are eliminated or persist in low numbers in a latent infection. Suppression of the immune system at a later date can result in reactivation of bacterial multiplication and the continuation of the *M. tuberculosis* infectious cycle.

immunological record of exposure to infection, remain incapable of fully controlling bacillary proliferation. Following the emergence of a productive Th-1-type cell-mediated immune response, macrophages become activated and proficient in killing bacilli. Fortunately, the majority of cases actually resolve without clinical tuberculosis (48). However, in a minority of individuals, the immune response to *M. tuberculosis* infection is suboptimal, allowing the organism to replicate to large numbers where it causes primary active disease (48), a condition characterized by persistent fatigue, anorexia, progressive weight loss, low-grade fever, and production of a chronic, often contagious, cough. However, more commonly, the organisms persist in low numbers in a poorly understood dormant or quiescent state termed latency (82). In contrast

to individuals suffering from acute infection, individuals latently infected with *M. tuberculosis* do not exhibit overt signs of disease, although they typically test positive for a delayed-type hypersensitivity response.

The latent infection is one of the least understood aspects of tuberculosis, despite the fact that it plays a crucial role in the propagation of tuberculosis and maintenance of effective infectious cycles in human populations (97). Immunocompetent individuals latently infected with *M. tuberculosis* carry a 2–23% *lifetime* risk of developing reactivation tuberculosis at a later date (97). However, individuals that are co-infected with the human immunodeficiency virus, or whose immune systems are suppressed due to other factors, carry a 5–10% *annual* risk of tuberculosis reactivation (97). The factors that influence the initial ability of the tubercle bacilli to replicate to large numbers, or alternatively establish persistent infection with a chance of reactivation at a later date, remain largely unknown. However, it has long been suggested that these factors may be intimately associated with the immune status of the host. Whereas *M. tuberculosis* readily grows in macrophages of immunocompromised hosts, macrophages of immunocompetent hosts are able to control bacterial proliferation (48). One mechanism utilized by the host to control *M. tuberculosis* multiplication and prevent further hematogenous dissemination of the organism to other sites is the formation of granulomas, areas of activated macrophages, $CD4^{+}$, and $CD8^{+}$ T cells that surround infected tissues (96). The activation of macrophages within granulomatous lesions is thought to be a key mechanism for control of *M. tuberculosis* infection, due in large part to the ability of activated macrophages to induce production of ROI and RNI. Not surprisingly, the significance of *nos2* and *phox* expression for this process has generated considerable attention.

ROLE OF RNI AND ROI IN TUBERCULOSIS

There is considerable evidence suggesting that expression of iNOS and generation of RNI play a significant role in the control of *M. tuberculosis* infection (Table 2). Inhibition of iNOS by chemical inhibitors such as aminoguanidine or *N*-monomethyl L-arginine exacerbates *M. tuberculosis* infection in mice using either the acute or low-dose persistent model of tuberculosis (15, 41). For example, mice treated with one of the NOS inhibitors shortly before or during the course of infection with *M. tuberculosis* succumb to disease within 34 days, whereas *M. tuberculosis*-infected mice receiving no treatment, or noninfected mice receiving treatment, survive for >120 days (15). Similarly, the addition of aminoguanidine to mice persistently infected with *M. tuberculosis* results in rapid disease reactivation and an increased bacillary burden shortly upon administration (41). Interestingly, the rate and extent of disease progression, as well as the pathological responses to iNOS inhibition during both the acute and chronic stages of infection, differ between the various organs examined. For example, immunohistochemical staining reveals that more iNOS is detected in the liver of aminoguanidine-treated animals relative to control animals, compared with the amount of iNOS detected in the lungs of aminoguanidine-treated mice relative to control mice (13, 41). Because the lungs of mice are generally considered more susceptible to *M. tuberculosis* infection compared with other organs (92), these observations suggest that murine tissues may differ in their innate susceptibility to *M. tuberculosis* infection, due in part to the amount of iNOS expressed.

Apart from *M. tuberculosis* infections carried out in the presence of NOS inhibitors, *M. tuberculosis* infections carried out using *nos2*$^{-/-}$ mice provide further evidence to suggest a role for RNI production in control of *M. tuberculosis* infection (Table 2). *nos2*$^{-/-}$ mice are highly susceptible to infection by *M. tuberculosis* and behave in a manner similar to that observed with *M. tuberculosis*-infected wild-type littermates receiving immune-suppressing glucocorticoid therapy (1, 72). For example, whereas *nos2*$^{-/-}$ mice infected with *M. tuberculosis* survive an average of 35–45 days, parental control mice (either heterozygous or homozygous wild type) survive an

TABLE 2. EFFECT OF RNI AND ROI INHIBITION ON *MYCOBACTERIUM TUBERCULOSIS* VIRULENCE

	*Method**	*Function*	*Host†*	*Infection stage*	*Effect*
RNI					
	AG	iNOS inhibitor	C57BL/6 mice	Acute	Decreased survival time from 120 to 34 days
	NMMA	NOS inhibitor	C57BL/6 mice	Acute	Decreased survival time from 120 to 38 days
	AG	iNOS inhibitor	C57BL/6 mice	Persistent	Increased bacillary burden and granuloma reaction
	NOS2$^{-/-}$	—	C57BL/6 mice	Acute	Decreased survival time from 160 to 45 days
	D-NIL	iNOS inhibitor	C57BL/6 mice	Persistent	Decreased survival time from >90 days to 65 days
	NOS2$^{-/-}$	—	Murine macrophages	—	Increased bacillary proliferation
	NOS2$^{-/-}$	—	B6 mice	Acute	Decreased survival time from 180 to 120 days
	NMMA	NOS inhibitor	Human AM	—	Increased bacillary proliferation
ROI					
	Catalase	ROI scavenger	Murine macrophages	—	No effect
	SOD	ROI scavenger	Murine macrophages	—	No effect
	Mannitol	ROI scavenger	Murine macrophages	—	No effect
	Diazabicyclooctane	ROI scavenger	Murine macrophages	—	No effect
	D9 ROI-deficient	—	Murine macrophages	—	No effect
	gp47^{phox}	—	C57BL/6 mice	Acute	Increased bacillary burden during early infection
	gp91^{phox}	—	Murine macrophages	—	No effect
	gp91^{phox}	—	B6 mice	Acute	Increased bacillary burden in some organs
	Phox$^{-/-}$	—	Human CGD macrophages	—	No effect

*RNI or ROI was inhibited by the addition of chemical inhibitors or by the use of transgenic mice mutant in *nos2* or components of *phox*. AG, aminoguanidine; AM, alveolar macrophages; NMMA, *N*-monomethyl L-arginine; NOS2$^{-/-}$ mice, iNOS-deficient mice; D-NIL, N^6-(1-iminoethyl)lysine; SOD, superoxide dismutase; gp47^{phox}, gp91^{phox}, and Phox$^{-/-}$, Phox-deficient mice.

†Tissue or animal model system used to evaluate *M. tuberculosis* virulence.

average of 150–160 days after *M. tuberculosis* infection (72). This difference in survival between *nos2*$^{-/-}$ and control mice directly correlates with the ability of the host to form productive RNI-expressing granulomas. *M. tuberculosis*-infected *nos2*$^{-/-}$ mice display large, sometimes necrotizing granulomatous lesions in their lungs, liver, and spleen prior to their death, concomitant with large numbers of acid-fast bacilli in these tissues (72), whereas *M. tuberculosis*-infected control mice killed at similar times display small granulomatous lesions in these tissues and harbor few acid-fast bacilli (72). In addition, high plasma nitrite and nitrate levels are observed in *M. tuberculosis*-infected tissues of control mice, but not in tissues obtained from *M. tuberculosis*-infected *nos2*$^{-/-}$ mice (72). Furthermore, the addition of other iNOS effector molecules, such as tumor necrosis factor-α neutralizing antibodies, results in a fatal reactivation of latent tuberculosis when administered to control mice persistently infected with *M. tuberculosis* (80). Taken together, these results strongly support a role for NOS in the control of *M. tuberculosis* in the murine model of tuberculosis.

Although no human genetic deficiency in *nos2* has been identified to date, iNOS expression and production of RNI are also likely to be relevant in the control of *M. tuberculosis* during human tuberculosis. For example, a significantly higher percentage of alveolar macrophages obtained from *M. tuberculosis*-infected patients (a) stain positive for diaphorase (a marker of nitrate levels), and (b)

stain positive with an antibody that recognizes iNOS, compared with staining of alveolar macrophages obtained from the lungs of healthy individuals (86). In addition, alveolar macrophages from patients with pulmonary fibrosis, but not from patients with lung cancer or pulmonary nodules, exhibit increased *nos2* mRNA expression and iNOS protein levels, and stain positive for peroxynitrite following infection with *M. bovis* BCG *in vitro* (89). However, the differences in virulence often observed between various clinical isolates of *M. tuberculosis* during infection do not coincide with an ability to modulate the amount of nitric oxide generated within the macrophage. For example, a panel of virulent *M. tuberculosis* strains ellicit similar levels of RNI as measured by nitrite accumulation following infection of macrophages, although these strains differ in their susceptibility to RNI generated in bacterial medium (100). In addition, there appears to be no correlation between the tolerance of a *M. tuberculosis* strain for RNI generated in a cell-free system and its ability to withstand RNI generated in macrophages primed with interferon-γ (100), or RNI generated during infection *in vivo* (91). Rather, more virulent isolates of *M. tuberculosis* may simply be less likely to expose themselves to RNI, as might be observed if some isolates were more effective at altering their trafficking to late-endosomal compartments (26).

As with iNOS and RNI generation, the ability of host macrophages to control *M. tuberculosis* infection by generation of ROI from NADPH oxidase remains controversial (Table 2). It has generally been thought that *M. tuberculosis* and other pathogenic mycobacteria are largely resistant to effects of ROI due in part to their ability to induce expression of a number of antioxidant activities, including superoxide dismutase and catalase. For example, *Mycobacterium microti* killing in murine macrophages *in vitro* is inhibited by addition of the H_2O_2 scavenger catalase (130). Also, experimental *M. tuberculosis* infection in *phox*-deficient mice results in an increase in bacillary burden prior to the emergence of adaptive immunity, and a resulting alteration in granuloma formation when compared with *M. tuberculosis* infections in control mice following the emergence of adaptive immunity (21). However, other investigators have reported little, if any, effect of NADPH oxidase-generated ROI on host–pathogen interactions involving *M. tuberculosis* (Table 2). For example, although activated murine macrophages are able to control *M. tuberculosis* and *M. avium* growth *in vitro,* the addition of reactive oxygen scavengers such as catalase, superoxide dismutase, mannitol, or diazabicyclooctane to these mycobacteria-infected macrophages fails to abolish the growth restriction (2, 25). Similarly, no significant difference in growth of *M. tuberculosis* is observed in ROI-deficient murine macrophage cell lines compared with parent ROI-generating J774.16 macrophages (14). Adding to this confusion has been the observation of an iNOS-independent antimycobacterial activity that may be mediated by ROI (7), and the observation that compounds such as aminoguanidine also inhibit ROI production in addition to RNI (7, 125). The lack of convincing evidence for a role of *phox*-mediated ROI production in control of *M. tuberculosis* infection appears to be applicable to human tuberculosis as well, as researchers studying the antimycobacterial capability of respiratory burst-deficient macrophages derived from CGD patients failed to observe any difference in *M. tuberculosis* growth following infection compared with macrophages obtained from healthy control individuals (40).

M. tuberculosis RESISTANCE MECHANISMS TO ROI AND RNI

Because of the experimental evidence suggesting a role for ROI and RNI in the control of *M. tuberculosis* infection, the identification of determinants allowing *M. tuberculosis* to resist and survive during periods of acute and persistent infection in the host has largely focused on defining components of oxidative and nitrosative stress response and elucidating their role in the infection process. The study of these pathways in *M. tuberculosis* and other mycobacterial species suggests that although effects resulting from exposure to

ROI and RNI are likely to be similar to those observed in enteric organisms, the regulation of these determinants differs substantially from the enteric paradigm. For example, exposure of *M. tuberculosis* to a wide range of H_2O_2 concentrations results in minimal (27, 44, 68, 112) differential gene expression during growth *in vitro,* whereas exposure of *E. coli* or *Salmonella* to similar H_2O_2 concentrations results in alteration of >40 gene products (81, 126). Similarly, treatment with cumene hydroperoxide or menadione induces the expression of only a few peptides in *M. tuberculosis* (44), whereas exposure of enteric organisms to these compounds induces a multigene response (49, 81, 131). Even the Fe-containing superoxide dismutase is not differentially induced in *M. tuberculosis* in response to menadione, a generator of superoxide (44), although this peptide is significantly induced in enteric bacteria in response to the same stress (49). The difference between mycobacteria and enteric bacteria in their response to oxidative and nitrosative stress can be attributed in part to mutations that render parts of the oxidative and nitrosative stress dysfunctional in mycobacterial species (Fig. 3). For example, studies of components of oxidative stress response have revealed the following: (a) the presumed central regulator of peroxidative and nitrosative stress response in *M. tuberculosis, oxyR,* is a pseudogene and contains multiple frameshift mutations and deletions that render it inactive (27, 112); (b) the other branches of the oxidative stress response, *furA* and *katG,* in the other major mycobacterial human pathogen, *Mycobacterium leprae,* are vestigial and also carry multiple mutations (29, 83, 94); and (c) the *soxRS* regulon appears to be absent from the genomes of mycobacterial species (19, 20).

All members of the *M. tuberculosis* complex (*M. tuberculosis, Mycobacterium bovis, Mycobacterium africanum,* and *Mycobacterium microti*) that have been examined carry similar mutations in *oxyR* (27, 112). In addition, other evolutionary distraught, fast-growing mycobacterial species, including *Mycobacterium smegmatis* and *Mycobacterium aurum,* lack *oxyR* in their genomes (30). Intact *oxyR* has been observed in a variety of mycobacterial species including *M. leprae, Mycobacterium marinum,* and *Mycobacterium avium* (94), suggesting that this regulator and its regulon are conserved and may still be important for aspects of physiology and/or virulence in the majority of members of the genus *Mycobacterium*. For example, purified OxyR from *M.*

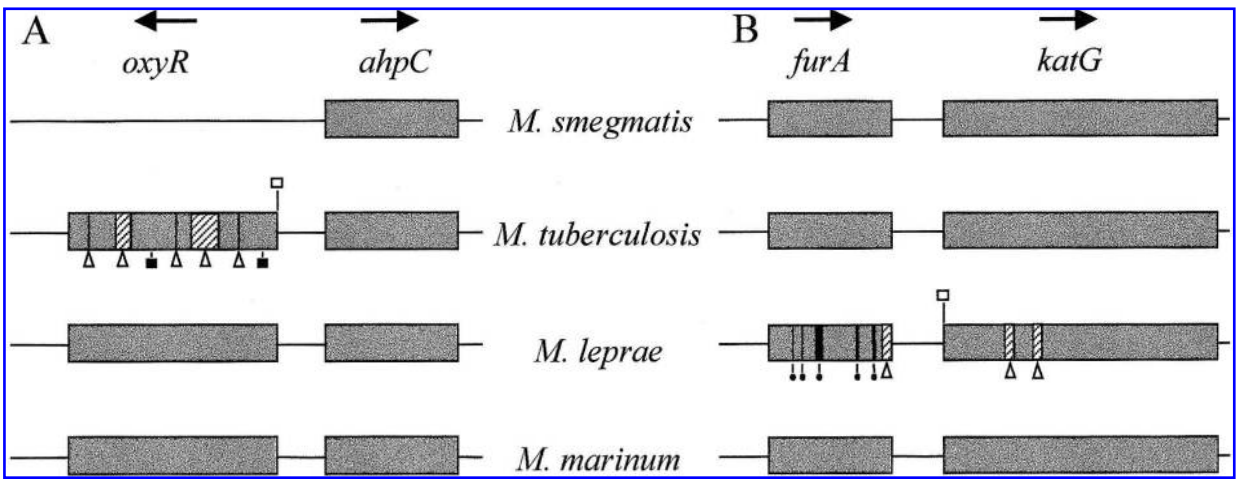

FIG. 3. Components of the oxidative stress response in Mycobacteria. Two branches of oxidative stress response exist in mycobacterial species. (**A**) The central regulator of the peroxide stress response is dysfunctional in the major human pathogen *Mycobacterium tuberculosis* due to the presence of multiple mutations and frameshift insertions. (**B**) The other branch of oxidative stress response encoded by regulator *furA* and catalase-peroxidase *katG* is dysfunctional in the other major human pathogen *Mycobacterium leprae.* Arrows, direction of transcription; open triangles below hatched segments, large deletions; triangles below lines, frameshift mutations; filled balloons, frameshift insertions; open squares, mutations in the start codon; filled squares, nonsense mutations.

leprae binds the *oxyR-ahpC* intergenic region in *M. leprae, M. marinum, Mycobacterium xenopi, Mycobacterium intracellulare,* and *M. tuberculosis* through recognition of ATC-N_9-GAT, a motif containing the T-N_{11}-A core characteristic of all LysR-type transcriptional regulators to which OxyR belongs (94). In addition, *oxyR* appears to regulate expression of *ahpC* in *M. marinum* following exposure to organic peroxides, a phenomenon that is lost following disruption of *oxyR* (94; Pagan-Ramos, unpublished observations). Although loss of *oxyR* in members of the *M. tuberculosis* complex, but not in other pathogenic mycobacterial species, appears paradoxical, we speculate that *oxyR* may have been lost from the tubercle bacilli to prevent expression of certain downstream effector determinants that may act as immunogens during stages of the infection process *in vivo* (29, 119). For example, AhpC is a close homolog of the natural killer cell enhancement factor NKEF known to stimulate natural killer cell cytotoxicity (109, 111), and is a well recognized immunogen in a number of pathogens including mycobacteria (90). The expression of *ahpC* in *M. tuberculosis* and *M. bovis* species is low or undetectable during growth *in vitro* and during intracellular growth in macrophages, whereas *ahpC* expression is high under these conditions in *M. bovis* BCG, an attenuated vaccine strain of *M. bovis* that is able to infect, but not establish, productive persistent infections in the host (119). In addition, although *ahpC* mutants of *M. tuberculosis* are not attenuated for growth during the acute phase of infection in the murine model of tuberculosis (119), AhpC may be required during later stages of the infection process (119). For example, *ahpC* expression is induced in *M. tuberculosis* during growth of the tubercle bacilli under static conditions (119), an *in vitro* condition thought to mimic the one *M. tuberculosis* encounters during growth in granulomatous lesions (132). Others have reported that expression of antisense RNA to *ahpC* does result in a marked attenuation in virulence in *M. bovis* when administered to guinea pigs (135). However, it is difficult to interpret these results in lieu of a *bona fide ahpC* knockout mutation.

A different component of oxidative stress response also appears to be dysfunctional in the other major human mycobacterial pathogen, *M. leprae.* In this organism, the *furA* and *katG* genes contain multiple mutations that render them both inactive (Fig. 3) (29, 83, 94). In a variety of organisms, Fur or Fur homologs regulate parts of oxidative stress response including superoxide dismutase (31, 88, 123), DNA repair enzymes (69), catalase and peroxidase genes (3, 10, 52, 127, 128), alkylhydroperoxidase genes (10, 127, 128), the *soxRS* regulon (141), and the *oxyR* gene (141). In all mycobacterial species that have been examined to date, *furA* lies immediately upstream of and is transcribed in the same direction as the catalase-peroxidase *katG* (137). Studies of these genes in *M. tuberculosis* (99) or in *M. smegmatis* (137), a species that also lacks *oxyR,* suggest that *furA* is a negative regulator of *katG* expression. For example, *M. smegmatis* strains disrupted in *furA* are more resistant to H_2O_2 than their wild-type parent, due to increased expression of *katG* (137). A similar mechanism of catalase-peroxidase regulation has also been observed in related species, including *Streptomyces coelicolor* and *Streptomyces reticuli* (51, 142). Although important for processes involving detoxification of peroxides *in vitro* (137), regulation of *katG* by FurA may also contribute to virulence in the tubercle bacilli. For example, *furA* and *katG* promoters are sequentially induced during growth of *M. tuberculosis* in murine macrophages (76). In addition, laboratory and clinical strains expressing little or no KatG are relatively sensitive to killing by exogenously added H_2O_2 (74) and during growth in human monocytes following stimulation of an endogenous oxidative burst (74). Furthermore, growth and persistence of a *katG*-deleted (INH^r) mutant of *M. tuberculosis* are severely compromised in both the murine and guinea pig models of tuberculosis (70), and introduction of wild-type copies of *furA* and *katG* into strains of *M. tuberculosis* deleted for these genes improves survival in the murine host (99). Thus, conservation of at least one branch of the oxidative stress response in the two frank human pathogens *M. tuberculosis* and *M. leprae* may be necessary

for the establishment or maintenance of infection *in vivo*.

Additional genes expressed in mycobacterial species may also contribute to oxidative and/or nitrosative stress response, and participate in aspects of virulence during the later stages of the infection process. In *M. smegmatis* and *M. tuberculosis,* an iron-dependent homolog of the diphtheria toxin repressor, *dtxR,* affects, directly or indirectly, expression of genes involved in ROI detoxification including *katG* and *sodA* (33, 34). *M. smegmatis ideR* mutants are more sensitive to H_2O_2 *in vitro* and exhibit decreased levels of catalase-peroxidase and manganese superoxide dismutase compared with wild-type strains (34). Introduction of a plasmid expressing the iron-independent, hyperrepressor mutant of DtxR, DtxR(E175K), attenuates virulence of *M. tuberculosis* in a murine model of tuberculosis during stages of persistent infection (73). Two additional genes from *M. tuberculosis* have also been shown to confer resistance to ROI and RNI in surrogate hosts, although their role in *M. tuberculosis* physiology remains unknown (36, 106). Deletion of one of these, *noxR1,* in *M. bovis* BCG results in increased sensitivity to *in vitro* generated RNI in comparison with the wild-type strain (120), although deletion of this gene in *M. tuberculosis* does not increase sensitivity to RNI *in vitro* or attenuate virulence of *M. tuberculosis in vivo* (120).

OXIDATIVE AND NITROSATIVE STRESS RESPONSE AND SUSCEPTIBILITY TO THE ANTITUBERCULOSIS DRUG INH

Apart from the putative role of oxidative and nitrosative resistance components in *M. tuberculosis* physiology and virulence, expression (or lack thereof) of these components may also play a role in the innate sensitivity and acquired resistance of *M. tuberculosis* to the front-line antitubercular drug isonicotinic acid hydrazide (isoniazid; INH) (Fig. 4). Although nearly 50 years have passed since the first report of INH in the treatment of tuberculosis (78), not until recently have mechanisms of INH action and/or resistance been elucidated. *M. tuberculosis* and other members of the *M. tuberculosis* complex are highly susceptible to INH, (in the range of 0.02–0.2 μg/ml) (110), whereas most other mycobacteria, including *M. leprae, M. marinum, M. avium,* and *M. smegmatis,* remain relatively resistant to the action of this drug (in the range of 1–10 μg/ml) (110). INH activation from its pro-drug form into its active antituberculosis form requires modification (possibly peroxidative) by KatG (138). In its active form, INH interferes with multiple processes including synthesis of essential cell-wall components and maintenance of appropriate NADH/NAD^+ intracellular ratios (28, 57). However, activation of INH from its pro-drug form into its active form is also thought to result in production of ROI (117, 118). The presence of a functional *katG* in *M. tuberculosis,* in combination with lack of functional *oxyR*-dependent gene induction, may explain in part the exquisite sensitivity of *M. tuberculosis* to INH. For example, mutations in the *oxyR* genes of *E. coli* and *S. typhimurium* (102), as well as *M. marinum* (Pagan-Ramos *et al.,* unpublished observations), render these normally resistant organisms sensitive to INH. The significance of dysfunctional components of oxidative

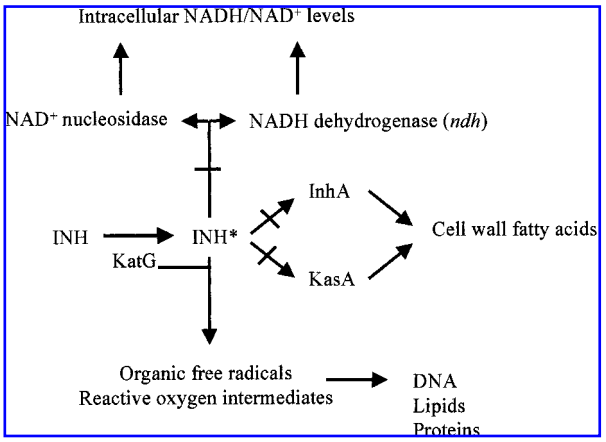

FIG. 4. Pathway of INH activation and targets of its activity in *Mycobacterium tuberculosis*. Activation of INH to its pro-drug form requires modification by KatG and generates organic free radicals and ROI. Activated INH inhibits the activity of cell-wall fatty acid machinery including fatty acid synthase II InhA and β-ketoacyl carrier protein synthase KasA. Activated INH also affects enzymes that modulate intracellular NADH/NAD^+ levels including NAD^+ nucleosidase and NADH dehydrogenase.

stress response may also contribute to acquired resistance to INH in *M. tuberculosis* and other members of the *M. tuberculosis* complex. For example, point mutations or deletions in *katG* are the most common mechanism of INH resistance in *M. tuberculosis* isolates recovered from diseased patients (56, 104). Isolates most resistant to INH tend to carry mutations that severely reduce or eliminate peroxidase activity (56, 139), whereas *M. tuberculosis* mutants that retain some degree of peroxidase activity remain moderately resistant to INH (56). In addition, introduction of *M. tuberculosis katG* into INH-resistant mutants of *M. tuberculosis* restores innate susceptibility to this drug (138), and derepression of *katG* expression by inactivation of negative regulator *furA* leads to increased sensitivity of mycobacteria to INH (137).

The inability to express *ahpC* in response to peroxide stress may also contribute to the extreme sensitivity and acquired resistance of *M. tuberculosis* to INH. *M. tuberculosis* and all members of the *M. tuberculosis* complex, in contrast to most other mycobacterial species examined, express low or undetectable levels of AhpC and are highly sensitive to INH (119, 140). In contrast, many clinical isolates resistant to INH carry mutations in the *ahpC* promoter region, resulting in increased alkylhydroperoxidase activity (30, 112, 134). Introduction of *ahpC* promoter-up mutations from an INH-resistant derivative of *M. bovis* into *M. smegmatis* also increases resistance to INH (136), and disruption of *ahpC* by gene inactivation increases sensitivity of *M. smegmatis* to INH (140). However, unlike the direct role for KatG activity in INH susceptibility, it remains unclear whether the mechanism of INH resistance via AhpC is direct or indirect. For example, increased levels of ROI resulting from loss of KatG-dependent catalase and peroxidase activity, or from metabolism of INH, may result in compensatory promoter-up mutations in *ahpC,* a characteristic often observed in INH-resistant isolates (30, 113, 114).

Primary targets for KatG-activated INH are components of the cell-wall biosynthesis machinery, including those encoded by *inhA* and *kasA* (4, 77). InhA is a component of fatty acid synthase II, a key enzyme in the pathway generating mycolic acids and other essential fatty acids of the mycobacterial cell-wall structure (107). Specifically, *inhA* catalyzes the NADH-dependent reduction of long-chain fatty acids (75), a process that can be inhibited *in vitro* using purified InhA, ^{14}C-labeled INH, NAD or NADH, KatG, and other minor components (62). *inhA* was first identified during a search for genes from INH-resistant mycobacteria that conferred resistance to INH-sensitive mycobacteria (4). Mutations in *inhA* account for INH resistance in a moderate number of clinical isolates of *M. tuberculosis* recovered from diseased humans (105).

In addition to InhA, activated INH also targets other components of the cell-wall biosynthesis machinery. Sequence analysis of the *kasA* gene from a genetically diverse panel of INH-resistant and INH-sensitive organisms indicates that mutations in this gene are common and occur in 5–20% of strains resistant to INH (77). *kasA* encodes a β-ketoacyl carrier protein synthase. In *M. tuberculosis, kasA* is induced in response to INH (134) and is able to confer resistance to INH-susceptible strains of *M. tuberculosis* when mutated (77). Mutations in this gene are not found in INH-sensitive strains, although a small subset of INH-resistant strains carry mutations in both *katG* and *kasA,* and as a result have a disproportionately high minimum inhibitory concentration for INH (77). Finally, KatG-dependent activation of INH may also alter other important cellular processes that result in INH resistance, including the intracellular NADH/NAD$^+$ levels. For example, INH may directly act to deplete NAD pools by inhibiting the NAD$^+$ nucleosidase, an enzyme responsible for the conversion of NAD to nicotinamide (6, 64). In addition, *M. smegmatis* strains carrying ts mutations in *ndh,* encoding a type II NADH dehydrogenase that oxidizes NADH and transfers electrons to quinones of the respiratory chain, are able to resist killing by high concentrations of INH (>100 μg/ml) (79). Mutations in additional *M. tuberculosis* genes may also confer INH resistance by altering NADH/NAD$^+$ ratios, although their mechanism is less clear (16).

CONCLUDING REMARKS

Resisting the effects of oxidative and nitrosative stress are processes critical for productive establishment and maintenance of infection by many bacterial pathogens. For *Mycobacterium tuberculosis* and other pathogenic mycobacterial species, these processes appear to differ from those observed in other intracellular bacteria such as enteric organisms. The continued study of *M. tuberculosis* and its oxidative and nitrosative stress response systems will continue to advance our knowledge regarding the multifaceted life cycle of this organism, as well as improve our understanding of adaptation processes required for survival during periods of acute and persistent infection.

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ABBREVIATIONS

CGD, chronic granulomatous disease; H_2O_2, hydrogen peroxide; INH, isonicotinic acid hydrazide, isoniazid; iNOS, inducible nitric oxide synthase; ·NO, nitric oxide; $\cdot NO_2$, nitrogen dioxide; NO_2^-, nitrite; NO_3^-, nitrate; NOS, nitric oxide synthase; *nos2,* inducible nitric oxide synthase; $O_2^-\cdot$, superoxide; ·OH, hydroxyl radical; $OONO^-$, peroxynitrite; Phox, NADPH oxidase; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates.

REFERENCES

1. Adams LB, Dinauer MC, Morgenstern DE, and Krahenbuhl JL. Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to *Mycobacterium tuberculosis* using transgenic mice. *Tuber Lung Dis* 78: 237–246, 1997.
2. Appelberg R and Orme IM. Effector mechanisms involved in cytokine-mediated bacteriostasis of *Mycobacterium avium* infections in murine macrophages. *Immunology* 80: 352–359, 1993.
3. Baillon ML, van Vliet AH, Ketley JM, Constantinidou C, and Penn CW. An iron-regulated alkyl hydroperoxide reductase (AhpC) confers aerotolerance and oxidative stress resistance to the microaerophilic pathogen *Campylobacter jejuni. J Bacteriol* 181: 4798–4804, 1999.
4. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, and Jacobs WR Jr. *inhA,* a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis. Science* 263: 227–230, 1994.
5. Barrett JF, Goldschmidt RM, Lawrence LE, Foleno B, Chen R, Demers JP, Johnson S, Kanojia R, Fernandez J, Bernstein J, Licata L, Donetz A, Huang S, Hlasta DJ, Macielag MJ, Ohemeng K, Frechette R, Frosco MB, Klaubert DH, Whiteley JM, Wang L, and Hoch JA. Antibacterial agents that inhibit two-component signal transduction systems. *Proc Natl Acad Sci U S A* 95: 5317–5322, 1998.
6. Bekierkunst A. Nicotinamide-adenine dinucleotide in tubercle bacilli exposed to isoniazid. *Science* 152: 525–526, 1966.
7. Bekker L-G, Freeman S, Murray PJ, Ryffel B, and Kaplan G. TNF-α controls intracellular mycobacterial growth by both inducible nitric oxide synthase-dependent and inducible nitric oxide synthase-independent pathways. *J Immunol* 166: 6728–6734, 2001.
8. Bloom BR and Murray CJ. Tuberculosis: commentary on a reemergent killer. *Science* 257: 1055–1064, 1992.
9. Bryk R, Griffin P, and Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* 407: 211–215, 2000.
10. Bsat N, Herbig A, Casillas-Martinez L, Setlow P, and Helmann JD. *Bacillus subtilis* contains multiple Fur homologues: identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. *Mol Microbiol* 29: 189–198, 1998.
11. Buchmeier NA, Lipps CJ, So MY, and Heffron F. Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. *Mol Microbiol* 7: 933–936, 1993.
12. Candeias LP, Patel KB, Stratford MR, and Wardman P. Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. *FEBS Lett* 333: 151–153, 1993.
13. Chan J and Kaufmann SHE. Immune mechanisms of protection. In: *Tuberculosis: Pathogenesis, Protection and Control,* edited by Bloom, BR. Washington, D.C.: American Society for Microbiology, 1994, pp. 389–415.
14. Chan J, Xing Y, Magliozzo RS, and Bloom BR. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by acti-

vated murine macrophages. *J Exp Med* 175: 1111–1122, 1992.
15. Chan J, Tanaka K, Carroll D, Flynn J, and Bloom BR. Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis*. *Infect Immun* 63: 736–740, 1995.
16. Chen L, Xie QW, and Nathan C. Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates. *Mol Cell* 1: 795–805, 1998.
17. Choi H, Kim S, Mukhopadhyay P, Cho S, Woo J, Storz G, and Ryu S. Structural basis of the redox switch in the OxyR transcription factor. *Cell* 105: 103–113, 2001.
18. Christman MF, Storz G, and Ames BN. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. *Proc Natl Acad Sci U S A* 86: 3484–3488, 1989.
19. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE III, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream M-A, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, and Barrell BG. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393: 537–544, 1998.
20. Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honore N, Garnier T, Churcher C, Harris D, Mungall K, Basham D, Brown D, Chillingworth T, Connor R, Davies RM, Devlin K, Duthoy S, Feltwell T, Fraser A, Hamlin N, Holroyd S, Hornsby T, Jagels K, Lacroix C, Maclean J, Moule S, Murphy L, Oliver K, Quail MA, Rajandream MA, Rutherford KM, Rutter S, Seeger K, Simon S, Simmonds M, Skelton J, Squares R, Squares S, Stevens K, Taylor K, Whitehead S, Woodward JR, and Barrell BG. Massive gene decay in the leprosy bacillus. *Nature* 409: 1007–1011, 2001.
21. Cooper AM, Segal BH, Frank AA, Holland SM, and Orme IM. Transient loss of resistance to pulmonary tuberculosis in $p47^{phox-/-}$ mice. *Infect Immun* 68: 1231–1234, 2000.
22. De Groote MA and Fang FC. Antimicrobial properties of nitric oxide. In: *Nitric Oxide and Infection*, edited by Fang FC. New York: Kluwer/Plenum, 1999, pp. 231–261.
23. De Groote MA, Testerman T, Xu Y, Stauffer G, and Fang FC. Homocysteine antagonism of nitric oxide-related cytostasis in *Salmonella typhimurium*. *Science* 272: 414–417, 1996.
24. De Groote MA, Ochsner UA, Shiloh MU, Nathan C, McCord JM, Dinauer MC, Libby SJ, Vazquez-Torres A, Xu Y, and Fang FC. Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase. *Proc Natl Acad Sci U S A* 94: 13997–14001, 1997.
25. Denis M. Interferon-gamma-treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. *Cell Immunol* 132: 150–157, 1991.
26. Deretic V and Fratti RA. *Mycobacterium tuberculosis* phagosome. *Mol Microbiol* 31: 1603–1609, 1999.
27. Deretic V, Philipp W, Dhandayuthapani S, Mudd MH, Curcic R, Garbe T, Heym B, Via LE, and Cole ST. *Mycobacterium tuberculosis* is a natural mutant with an inactivated oxidative-stress regulatory gene: implications for sensitivity to isoniazid. *Mol Microbiol* 17: 889–900, 1995.
28. Deretic V, Pagan-Ramos E, Zhang Y, Dhandayuthapani S, and Via LE. The extreme sensitivity of *Mycobacterium tuberculosis* to the front-line antituberculosis drug isoniazid. *Nat Biotechnol* 14: 1557–1561, 1996.
29. Deretic V, Song J, and Pagan-Ramos E. Loss of *oxyR* in *Mycobacterium tuberculosis*. *Trends Microbiol* 5: 367–372, 1997.
30. Dhandayuthapani S, Zhang Y, Mudd MH, and Deretic V. Oxidative stress response and its role in sensitivity to isoniazid in mycobacteria: characterization and inducibility of *ahpC* by peroxides in *Mycobacterium smegmatis* and lack of expression in *M. aurum* and *M. tuberculosis*. *J Bacteriol* 178: 3641–3649, 1996.
31. Dubrac S and Touati D. Fur positive regulation of iron superoxide dismutase in *Escherichia coli*: functional analysis of the *sodB* promoter. *J Bacteriol* 182: 3802–3808, 2000.
32. Dukan S and Touati D. Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. *J Bacteriol* 178: 6145–6150, 1996.
33. Dussurget O and Smith I. Interdependence of mycobacterial iron regulation, oxidative-stress response and isoniazid resistance. *Trends Microbiol* 6: 354–358, 1998.
34. Dussurget O, Rodriguez M, and Smith I. An *ideR* mutant of *Mycobacterium smegmatis* has derepressed siderophore production and an altered oxidative-stress response. *Mol Microbiol* 22: 535–544, 1996.
35. Dye C, Scheele S, Dolin P, Pathania V, and Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* 282: 677–686, 1999.
36. Ehrt S, Shiloh MU, Ruan J, Choi M, Gunzburg S, Nathan C, Xie Q, and Riley LW. A novel antioxidant gene from *Mycobacterium tuberculosis*. *J Exp Med* 186: 1885–1896, 1997.
37. Fang FC. Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. *J Clin Invest* 99: 2818–2825, 1997.
38. Fang FC, Vazquez-Torres A, and Xu Y. The transcriptional regulator SoxS is required for resistance of *Salmonella typhimurium* to paraquat but not for virulence in mice. *Infect Immun* 65: 5371–5375, 1997.

39. Farrant JL, Sansone A, Canvin JR, Pallen MJ, Langford PR, Wallis TS, Dougan G, and Kroll JS. Bacterial copper- and zinc-cofactored superoxide dismutase contributes to the pathogenesis of systemic salmonellosis. *Mol Microbiol* 25: 785–796, 1997.
40. Fazal N. The role of reactive oxygen species (ROS) in the effector mechanisms of human antimycobacterial immunity. *Biochem Mol Biol Int* 43: 399–408, 1997.
41. Flynn JL, Scanga CA, Tanaka KE, and Chan J. Effects of aminoguanidine on latent murine tuberculosis. *J Immunol* 160: 1796–1803, 1998.
42. Foote CS, Goyne TE, and Lehrer RI. Assessment of chlorination by human neutrophils. *Nature* 301: 715–716, 1983.
43. Francis KP, Taylor PD, Inchley CJ, and Gallagher MP. Identification of the *ahp* operon of *Salmonella typhimurium* as a macrophage-induced locus. *J Bacteriol* 179: 4046–4048, 1997.
44. Garbe TR, Hibler NS, and Deretic V. Response of *Mycobacterium tuberculosis* to reactive oxygen and nitrogen intermediates. *Mol Med* 2: 134–142, 1996.
45. Gaudu P and Weiss B. SoxR, a [2Fe-2S] transcription factor, is active only in its oxidized form. *Proc Natl Acad Sci U S A* 93: 10094–10098, 1995.
46. Goldberg M, Belkowski LS, and Bloom BR. Regulation of macrophage function by interferon-gamma. Somatic cell genetic approaches in murine macrophage cell lines to mechanisms of growth inhibition, the oxidative burst, and expression of the chronic granulomatous disease gene. *J Clin Invest* 85: 563–569, 1990.
47. Goode HF, Cowley HC, Walker BE, Howdle PD, and Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. *Crit Care Med* 23: 646–651, 1995.
48. Grange JM *Mycobacteria and Human Disease.* London: Arnold, Edward, 1988.
49. Greenberg JT and Demple B. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. *J Bacteriol* 171: 3933–3939, 1989.
50. Greenberg JT, Monach P, Chou JH, Josephy PD, and Demple B. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc Natl Acad Sci U S A* 87: 6181–6185, 1990.
51. Hahn JS, Oh SY, and Roe JH. Regulation of the *furA* and *catC* operon, encoding a ferric uptake regulator homologue and catalase-peroxidase, respectively, in *Streptomyces coelicolor* A3(2). *J Bacteriol* 182: 3767–3774, 2000.
52. Hassett DJ, Howell ML, Ochsner UA, Vasil ML, Johnson Z, and Dean GE. An operon containing *fumC* and *sodA* encoding fumarase C and manganese superoxide dismutase is controlled by the ferric uptake regulator in *Pseudomonas aeruginosa*: *fur* mutants produce elevated alginate levels. *J Bacteriol* 179: 1452–1459, 1997.
53. Hausladen A, Privalle CT, Keng T, DeAngelo J, and Stamler JS. Nitrosative stress: activation of the transcription factor OxyR. *Cell* 86: 719–729, 1996.
54. Hausladen A, Gow AJ, and Stamler JS. Nitrosative stress: metabolic pathway involving the flavohemoglobin. *Proc Natl Acad Sci U S A* 95: 14100–14105, 1998.
55. Held AM, Halko DJ, and Hurst JK. Mechanisms of chlorine oxidation of hydrogen peroxide. *J Am Chem Soc* 100: 5732–5740, 1981.
56. Heym B, Alzari PM, Honore N, and Cole ST. Missense mutations in the catalase-peroxidase gene, *katG,* are associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol* 15: 235–245, 1995.
57. Heym B, Saint-Joanis B, and Cole ST. The molecular basis of isoniazid resistance in *Mycobacterium tuberculosis*. *Tuber Lung Dis* 79: 267–271, 1999.
58. Hurst JK, Carr PAG, Hovis FE, and Richardson RJ. Hydrogen peroxide oxidation by chlorine compounds. Reaction dynamics and singlet oxygen formation. *Inorg Chem* 20: 2435–2468, 1981.
59. Imlay JA and Linn S. DNA damage and oxygen radical toxicity. *Science* 240: 1302–1309, 1988.
60. Imlay JA, Chin SM, and Linn S. Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. *Science* 240: 640–642, 1988.
61. Jackson SH, Gallin JI, and Holland SM. The p47*phox* mouse knock-out model of chronic granulomatous disease. *J Exp Med* 182: 751–758, 1995.
62. Johnsson K, King DS, and Schultz PG. Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis. *J Am Chem Soc* 117: 5009–5010, 1995.
63. Kagaya K, Miyakawa Y, Watanabe K, and Fukazawa Y. Antigenic role of stress-induced catalase of *Salmonella typhimurium* in cell-mediated immunity. *Infect Immun* 60: 1820–1825, 1992.
64. Kasarov LB and Moat AG. Metabolism of nicotinamide adenine dinucleotide in human and bovine strains of *Mycobacterium tuberculosis*. *J Bacteriol* 110: 600–603, 1972.
65. Khan AU and Kasha M. Singlet molecular oxygen evolution upon simple acidification of aqueous hypochlorite: application to studies on the deleterious health effects of chlorinated drinking water. *Proc Natl Acad Sci U S A* 91: 12362–12364, 1994.
66. Khan AU and Kasha M. Singlet molecular oxygen in the Haber–Weiss reaction. *Proc Natl Acad Sci U S A* 91: 12365–12367, 1994.
67. Klebanoff SJ. Myeloperoxidase–halide–hydrogen peroxide antibacterial system. *J Bacteriol* 95: 2131–2138, 1968.
68. Lee BY and Horwitz MA. Identification of macrophage and stress-induced proteins of *Mycobacterium tuberculosis*. *J Clin Invest* 96: 245–249, 1995.
69. Lee HS, Lee YS, Kim HS, Choi JY, Hassan HM, and Chung MH. Mechanism of regulation of 8-hydroxyguanine endonuclease by oxidative stress: roles of FNR, ArcA, and Fur. *Free Radic Biol Med* 24: 1193–1201, 1998.

70. Li Z, Kelley C, Collins F, Rouse D, and Morris S. Expression of *katG* in *Mycobacterium tuberculosis* is associated with its growth and persistence in mice and guinea pigs. *J Infect Dis* 177: 1030–1035, 1998.
71. Lundberg BE, Wolf RE Jr, Dinauer MC, Xu Y, and Fang FC. Glucose 6-phosphate dehydrogenase is required for *Salmonella typhimurium* virulence and resistance to reactive oxygen and nitrogen intermediates. *Infect Immun* 67: 436–438, 1999.
72. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, and Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc Natl Acad Sci U S A* 94: 5243–5248, 1997.
73. Manabe YC, Saviola BJ, Sun L, Murphy JR, and Bishai WR. Attenuation of virulence in *Mycobacterium tuberculosis* expressing a constitutively active iron repressor. *Proc Natl Acad Sci U S A* 96: 12844–1 2848, 1999.
74. Manca C, Paul S, Barry CE 3rd, Freedman VH, and Kaplan G. *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. *Infect Immun* 67: 74–79, 1999.
75. Marrakchi H, Laneelle G, and Quemard A. InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II. *Microbiology* 146: 289–296, 2000.
76. Master S, Zahrt TC, Song J, and Deretic V. Mapping of *Mycobacterium tuberculosis katG* promoters and their differential expression in infected macrophages. *J Bacteriol* 183: 4033–4039, 2001.
77. Mdluli K, Slayden RA, Zhu Y, Ramaswamy S, Pan X, Mead D, Crane DD, Musser JM, and Barry CE 3rd. Inhibition of a *Mycobacterium tuberculosis* beta-ketoacyl ACP synthase by isoniazid. *Science* 280: 1607–1610, 1998.
78. Middlebrook G. Sterilization of tubercle bacilli by isonicotinic acid hydrazide and the incidence of variants resistant to the drug *in vitro*. *Am Rev Tuberc* 65: 765–767, 1952.
79. Miesel L, Weisbrod TR, Marcinkeviciene JA, Bittman R, and Jacobs WR Jr. NADH dehydrogenase defects confer isoniazid resistance and conditional lethality in *Mycobacterium smegmatis*. *J Bacteriol* 180: 2459–2467, 1998.
80. Mohan VP, Scanga CA, Yu K, Scott HM, Tanaka KE, Tsang E, Tsai MM, Flynn JL, and Chan J. Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. *Infect Immun* 69: 1847–1855, 2001.
81. Morgan RW, Christman MF, Jacobson FS, Storz G, and Ames BN. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. *Proc Natl Acad Sci U S A* 83: 8059–8063, 1986.
82. Murray PJ. Defining the requirements for immunological control of mycobacterial infections. *Trends Microbiol* 7: 366–372, 1999.
83. Nakata N, Matsuoka M, Kashiwabara Y, Okada N, and Sasakawa C. Nucleotide sequence of the *Mycobacterium leprae katG* region. *J Bacteriol* 179: 3053–3057, 1997.
84. Nathan C. Nitric oxide as a secretory product of mammalian cells. *Faseb J* 6: 3051–3064, 1992.
85. Nathan C and Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci U S A* 97: 8841–8848, 2000.
86. Nicholson S, Bonecini-Almeida M, Lapa e Silva JR, Nathan C, Xie QW, Mumford R, Weidner JR, Calaycay J, Geng J, and Boechat N. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *J Exp Med* 183: 2293–2302, 1996.
87. Nicholson SC, Grobmyer SR, Shiloh MU, Brause JE, Potter S, MacMicking JD, Dinauer MC, and Nathan CF. Lethality of endotoxin in mice genetically deficient in the respiratory burst oxidase, inducible nitric oxide synthase, or both. *Shock* 11: 253–258, 1999.
88. Niederhoffer EC, Naranjo CM, Bradley KL, and Fee JA. Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus. *J Bacteriol* 172: 1930–1938, 1990.
89. Nozaki Y, Hasegawa Y, Ichiyama S, Nakashima I, and Shimokata K. Mechanism of nitric oxide-dependent killing of *Mycobacterium bovis* BCG in human alveolar macrophages. *Infect Immun* 65: 3644–3647, 1997.
90. Olsen I, Reitan LJ, Holstad G, and Wiker HG. Alkyl hydroperoxide reductases C and D are major antigens constitutively expressed by *Mycobacterium avium* subsp. *paratuberculosis*. *Infect Immun* 68: 801–808, 2000.
91. Ordway DJ, Sonnenberg MG, Donahue SA, Belisle JT, and Orme IM. Drug-resistant strains of *Mycobacterium tuberculosis* exhibit a range of virulence for mice. *Infect Immun* 63: 741–743, 1995.
92. Orme IM and Collins FM. Mouse model of tuberculosis. In: Tuberculosis: Pathogenesis, Protection, and Control, edited by Bloom, BR. Washington, D.C.: American Society for Microbiology, 1994, pp. 113–134.
93. Pacelli R, Wink DA, Cook JA, Krishna MC, DeGraff W, Friedman N, Tsokos M, Samuni A, and Mitchell JB. Nitric oxide potentiates hydrogen peroxide-induced killing of *Escherichia coli*. *J Exp Med* 182: 1469–1479, 1995.
94. Pagan-Ramos E, Song J, McFalone M, Mudd MH, and Deretic V. Oxidative stress response and characterization of the *oxyR-ahpC* and *furA-katG* loci in *Mycobacterium marinum*. *J Bacteriol* 180: 4856–4864, 1998.
95. Papp-Szabo E, Firtel M, and Josephy PD. Comparison of the sensitivities of *Salmonella typhimurium oxyR* and *katG* mutants to killing by human neutrophils. *Infect Immun* 62: 2662–2668, 1994.
96. Parham P. *The Immune System*. New York: Garland Publishing/Elsevier Science Ltd., 2000.
97. Parrish NM, Dick JD, and Bishai WR. Mechanisms of latency in *Mycobacterium tuberculosis*. *Trends Microbiol* 6: 107–112, 1998.

98. Pollock JD, Williams DA, Gifford MA, Li LL, Du X, Fisherman J, Orkin SH, Doerschuk CM, and Dinauer MC. Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. *Nat Genet* 9: 202–209, 1995.
99. Pym AS, Domenech P, Honore N, Song J, Deretic V, and Cole ST. Regulation of catalase-peroxidase (KatG) expression, isoniazid sensitivity and virulence by *furA* of *Mycobacterium tuberculosis*. *Mol Microbiol* 40: 879–889, 2001.
100. Rhoades ER and Orme IM. Susceptibility of a panel of virulent strains of *Mycobacterium tuberculosis* to reactive nitrogen intermediates. *Infect Immun* 65: 1189–1195, 1997.
101. Rook GA and Hernandez-Pando R. The pathogenesis of tuberculosis. *Annu Rev Microbiol* 50: 259–284, 1996.
102. Rosner JL. Susceptibilities of *oxyR* regulon mutants of *Escherichia coli* and *Salmonella typhimurium* to isoniazid. *Antimicrob Agents Chemother* 37: 2251–2253, 1993.
103. Rotrosen D and Gallin JI. Disorders of phagocyte function. *Annu Rev Immunol* 5: 127–150, 1987.
104. Rouse DA and Morris SL. Molecular mechanisms of isoniazid resistance in *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *Infect Immun* 63: 1427–1433, 1995.
105. Rouse DA, Li Z, Bai GH, and Morris SL. Characterization of the *katG* and *inhA* genes of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 39: 2472–2477, 1995.
106. Ruan J, St John G, Ehrt S, Riley L, and Nathan C. *noxR3*, a novel gene from *Mycobacterium tuberculosis*, protects *Salmonella typhimurium* from nitrosative and oxidative stress. *Infect Immun* 67: 3276–3283, 1999.
107. Sacchettini JC and Blanchard JS. The structure and function of the isoniazid target in *M. tuberculosis*. *Res Microbiol* 147: 36–43, 1996.
108. Saran M, Beck-Speier I, Fellerhoff B, and Bauer G. Phagocytic killing of microorganisms by radical processes: consequences of the reaction of hydroxyl radicals with chloride yielding chlorine atoms. *Free Radic Biol Med* 26: 482–490, 1999.
109. Sauri H, Ashjian PH, Kim AT, and Shau H. Recombinant natural killer enhancing factor augments natural killer cytotoxicity. *J Leukoc Biol* 59: 925–931, 1996.
110. Seydel JK, Schaper KJ, Wempe E, and Cordes HP. Mode of action and quantitative structure–activity correlations of tuberculostatic drugs of the isonicotinic acid hydrazide type. *J Med Chem* 19: 483–492, 1976.
111. Shau H, Gupta RK, and Golub SH. Identification of a natural killer enhancing factor (NKEF) from human erythroid cells. *Cell Immunol* 147: 1–11, 1993.
112. Sherman DR, Sabo PJ, Hickey MJ, Arain TM, Mahairas GG, Yuan Y, Barry CE 3rd, and Stover CK. Disparate responses to oxidative stress in saprophytic and pathogenic mycobacteria. *Proc Natl Acad Sci U S A* 92: 6625–6629, 1995.
113. Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry CE 3rd, and Stover CK. Compensatory *ahpC* gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. *Science* 272: 1641–1643, 1996.
114. Sherman DR, Mdluli K, Hickey MJ, Barry CE 3rd, and Stover CK. AhpC, oxidative stress and drug resistance in *Mycobacterium tuberculosis*. *Biofactors* 10: 211–217, 1999.
115. Shiloh MU and Nathan CF. Reactive nitrogen intermediates and the pathogenesis of Salmonella and mycobacteria. *Curr Opin Microbiol* 3: 35–42, 2000.
116. Shiloh MU, MacMicking JD, Nicholson S, Brause JE, Potter S, Marino M, Fang F, Dinauer M, and Nathan C. Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase. *Immunity* 10: 29–38, 1999.
117. Shoeb HA, Bowman BU Jr, Ottolenghi AC, and Merola AJ. Enzymatic and nonenzymatic superoxide-generating reactions of isoniazid. *Antimicrob Agents Chemother* 27: 408–412, 1985.
118. Shoeb HA, Bowman BU Jr, Ottolenghi AC, and Merola AJ. Evidence for the generation of active oxygen by isoniazid treatment of extracts of *Mycobacterium tuberculosis* H37Ra. *Antimicrob Agents Chemother* 27: 404–407, 1985.
119. Springer B, Master S, Sander P, Zahrt T, McFalone M, Song J, Papavinasasundaram KG, Colston MJ, Boettger E, and Deretic V. Silencing of oxidative stress response in *Mycobacterium tuberculosis*: expression patterns of *ahpC* in virulent and avirulent strains and effect of *ahpC* inactivation. *Infect Immun* 69: 5967–5973, 2001.
120. Stewart GR, Ehrt S, Riley LW, Dale JW, and McFadden J. Deletion of the putative antioxidant *noxR1* does not alter the virulence of *Mycobacterium tuberculosis* H37Rv. *Tuber Lung Dis* 80: 237–242, 2000.
121. Storz G and Imlay JA. Oxidative stress. *Curr Opin Microbiol* 2: 188–194, 1999.
122. Storz G and Zheng M. Oxidative stress. In: *Bacterial Stress Response*, edited by Storz G and Hengge-Aronis R. Washington, D.C.: American Society for Microbiology, 2000, pp. 47–59.
123. Tardat B and Touati D. Iron and oxygen regulation of *Escherichia coli* MnSOD expression: competition between the global regulators Fur and ArcA for binding to DNA. *Mol Microbiol* 9: 53–63, 1993.
124. Taylor PD, Inchley CJ, and Gallagher MP. The *Salmonella typhimurium* AhpC polypeptide is not essential for virulence in BALB/c mice but is recognized as an antigen during infection. *Infect Immun* 66: 3208–3217, 1998.
125. Tian Y, Xing Y, Magliozzo R, Yu K, Bloom BR, and Chan J. A commercial preparation of catalase inhibits nitric oxide production by activated murine macrophages: role of arginase. *Infect Immun* 68: 3015–3018, 2000.
126. VanBogelen RA, Kelley PM, and Neidhardt FC. Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. *J Bacteriol* 169: 26–32, 1987.

127. van Vliet AH, Baillon ML, Penn CW, and Ketley JM. *Campylobacter jejuni* contains two *fur* homologs: characterization of iron-responsive regulation of peroxide stress defense genes by the PerR repressor. *J Bacteriol* 181: 6371–6376, 1999.
128. van Vliet AHM, Wooldridge KG, and Ketley JM. Iron-responsive gene regulation in a *Campylobacter jejuni fur* mutant. *J Bacteriol* 180: 5291–5298, 1998.
129. Vazquez-Torres A, Xu Y, Jones-Carson J, Holden DW, Lucia SM, Dinauer MC, Mastroeni P, and Fang FC. Salmonella pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. *Science* 287: 1655–1658, 2000.
130. Walker L and Lowrie DB. Killing of *Mycobacterium microti* by immunologically activated macrophages. *Nature* 293: 69–71, 1981.
131. Walkup LK and Kogoma T. *Escherichia coli* proteins inducible by oxidative stress mediated by the superoxide radical. *J Bacteriol* 171: 1476–1484, 1989.
132. Wayne LG. Dormancy of *Mycobacterium tuberculosis* and latency of disease. *Eur J Clin Microbiol Infect Dis* 13: 908–914, 1994.
133. Weinberg JB. Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. *Mol Med* 4: 557–591, 1998.
134. Wilson M, DeRisi J, Kristensen HH, Imboden P, Rane S, Brown PO, and Schoolnik GK. Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. *Proc Natl Acad Sci U S A* 96: 12833–12838, 1999.
135. Wilson T, de Lisle GW, Marcinkeviciene JA, Blanchard JS, and Collins DM. Antisense RNA to *ahpC,* an oxidative stress defence gene involved in isoniazid resistance, indicates that AhpC of *Mycobacterium bovis* has virulence properties. *Microbiology* 144: 2687–2695, 1998.
136. Wilson TM and Collins DM. *ahpC,* a gene involved in isoniazid resistance of the *Mycobacterium tuberculosis* complex. *Mol Microbiol* 19: 1025–1034, 1996.
137. Zahrt TC, Song J, Siple J, and Deretic V. Mycobacterial FurA is a negative regulator of catalase-peroxidase gene *katG*. *Mol Microbiol* 39: 1174–1185, 2001.
138. Zhang Y and Young DB. Molecular mechanisms of isoniazid: a drug at the front line of tuberculosis control. *Trends Microbiol* 1: 109–113, 1993.
139. Zhang Y, Heym B, Allen B, Young D, and Cole S. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 358: 591–593, 1992.
140. Zhang Y, Dhandayuthapani S, and Deretic V. Molecular basis for the exquisite sensitivity of *Mycobacterium tuberculosis* to isoniazid. *Proc Natl Acad Sci U S A* 93: 13212–13216, 1996.
141. Zheng M, Doan B, Schneider TD, and Storz G. OxyR and SoxRS regulation of *fur*. *J Bacteriol* 181: 4639–4643, 1999.
142. Zou P, Borovok I, Ortiz de Orue Lucana D, Muller D, and Schrempf H. The mycelium-associated *Streptomyces reticuli* catalase-peroxidase, its gene and regulation by FurS. *Microbiology* 145: 549–559, 1999.

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2. Ratna B. Gurung, Auriol C. Purdie, Douglas J. Begg, Richard J. Whittington. 2012. In silico screened Mycobacterium avium subsp. paratuberculosis (MAP) recombinant proteins upregulated under stress conditions are immunogenic in sheep. *Veterinary Immunology and Immunopathology* . [CrossRef]
3. Ahmad K. Mashmoushi, Gary S. Gilkeson, Jim C. OatesThe Role of Reactive Nitrogen and Oxygen Intermediates in Systemic Lupus Erythematosus 199-211. [CrossRef]
4. Sung-Hyun Park, Hyun-Wook Lee, Weiguo Cao. 2010. Screening of nitrosative stress resistance genes in Coxiella burnetii: Involvement of nucleotide excision repair. *Microbial Pathogenesis* **49**:6, 323-329. [CrossRef]
5. Satoko Kawaji, Ling Zhong, Richard J. Whittington. 2010. Partial proteome of Mycobacterium avium subsp. paratuberculosis under oxidative and nitrosative stress. *Veterinary Microbiology* **145**:3-4, 252-264. [CrossRef]
6. Kingston H.G. Mills, Aoife P. BoydEvasion of Immune Responses by Bacteria . [CrossRef]
7. Joseph Chao, Dennis Wong, Xingji Zheng, Valerie Poirier, Horacio Bach, Zakaria Hmama, Yossef Av-Gay. 2010. Protein kinase and phosphatase signaling in Mycobacterium tuberculosis physiology and pathogenesis. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1804**:3, 620-627. [CrossRef]
8. Richard W Stokes, Simon J Waddell. 2009. Adjusting to a new home: Mycobacterium tuberculosis gene expression in response to an intracellular lifestyle. *Future Microbiology* **4**:10, 1317-1335. [CrossRef]
9. Rodgoun Attarian, Chelsea Bennie, Horacio Bach, Yossef Av-Gay. 2009. Glutathione disulfide and S-nitrosoglutathione detoxification by Mycobacterium tuberculosis thioredoxin system. *FEBS Letters* **583**:19, 3215-3220. [CrossRef]
10. Md. Suhail Alam, Saurabh K. Garg, Pushpa Agrawal. 2009. Studies on structural and functional divergence among seven WhiB proteins of Mycobacterium tuberculosis H37Rv. *FEBS Journal* **276**:1, 76-93. [CrossRef]
11. Daniel Ågren, Robert Schnell, Gunter Schneider. 2009. The C-terminal of CysM from Mycobacterium tuberculosis protects the aminoacrylate intermediate and is involved in sulfur donor selectivity. *FEBS Letters* **583**:2, 330-336. [CrossRef]
12. Paolo Ascenzi, Paolo ViscaScavenging of Reactive Nitrogen Species by Mycobacterial Truncated Hemoglobins **436**, 317-337. [CrossRef]
13. Jan Jacobsen, Svend J. Knak Jensen. 2007. A mechanism for production of singlet oxygen by acidification of hypochlorite. *Chemical Physics Letters* **449**:1-3, 135-137. [CrossRef]
14. Deanna A. Hagge, Vilma T. Marks, Nashone A. Ray, Marilyn A. Dietrich, Michael T. Kearney, David M. Scollard, James L. Krahenbuhl, Linda B. Adams. 2007. Emergence of an effective adaptive cell mediated immune response to Mycobacterium leprae is not impaired in reactive oxygen intermediate-deficient mice. *FEMS Immunology & Medical Microbiology* **51**:1, 92-101. [CrossRef]
15. Paolo Ascenzi, Martino Bolognesi, Mario Milani, Michel Guertin, Paolo Visca. 2007. Mycobacterial truncated hemoglobins: From genes to functions. *Gene* **398**:1-2, 42-51. [CrossRef]
16. Paolo Ascenzi, Mario Milani, Paolo Visca. 2006. Peroxynitrite scavenging by ferrous truncated hemoglobin GlbO from Mycobacterium leprae#. *Biochemical and Biophysical Research Communications* **351**:2, 528-533. [CrossRef]
17. Jim C. Oates, Gary S. Gilkeson. 2006. The biology of nitric oxide and other reactive intermediates in systemic lupus erythematosus. *Clinical Immunology* **121**:3, 243-250. [CrossRef]

18. P AKHTAR, S SRIVASTAVA, A SRIVASTAVA, M SRIVASTAVA, B SRIVASTAVA, R SRIVASTAVA. 2006. Rv3303c of Mycobacterium tuberculosis protects tubercle bacilli against oxidative stress in vivo and contributes to virulence in mice. *Microbes and Infection* **8**:14-15, 2855-2862. [CrossRef]
19. J HODGSON, C WATKINS, C BAYNE. 2006. Contribution of respiratory burst activity to innate immune function and the effects of disease status and agent on chemiluminescence responses by ruminant phagocytes in vitro. *Veterinary Immunology and Immunopathology* **112**:1-2, 12-23. [CrossRef]
20. A MAETZKE, S KNAKJENSEN. 2006. Reaction paths for production of singlet oxygen from hydrogen peroxide and hypochlorite. *Chemical Physics Letters* **425**:1-3, 40-43. [CrossRef]
21. Elsa Anes, Pascale Peyron, Leila Staali, Luisa Jordao, Maximiliano G. Gutierrez, Holger Kress, Monica Hagedorn, Isabelle Maridonneau-Parini, Mhairi A. Skinner, Alan G. Wildeman, Stefanos A. Kalamidas, Mark Kuehnel, Gareth Griffiths. 2006. Dynamic life and death interactions between Mycobacterium smegmatis and J774 macrophages. *Cellular Microbiology* **8**:6, 939-960. [CrossRef]
22. Giulia Fabozzi, Paolo Ascenzi, Simona Di Renzi, Paolo Visca. 2006. Truncated hemoglobin GlbO from Mycobacterium leprae alleviates nitric oxide toxicity. *Microbial Pathogenesis* **40**:5, 211-220. [CrossRef]
23. W FLORIO, G BATONI, S ESIN, D BOTTAI, G MAISETTA, F FAVILLI, F BRANCATISANO, M CAMPA. 2006. Influence of culture medium on the resistance and response of Mycobacterium bovis BCG to reactive nitrogen intermediates. *Microbes and Infection* **8**:2, 434-441. [CrossRef]
24. P ASCENZI, A BOCEDI, M BOLOGNESI, G FABOZZI, M MILANI, P VISCA. 2006. Nitric oxide scavenging by Mycobacterium leprae GlbO involves the formation of the ferric heme-bound peroxynitrite intermediate#. *Biochemical and Biophysical Research Communications* **339**:1, 450-456. [CrossRef]
25. A ROOYAKKERS, R STOKES. 2005. Absence of complement receptor 3 results in reduced binding and ingestion of but has no significant effect on the induction of reactive oxygen and nitrogen intermediates or on the survival of the bacteria in resident and interferon-gamma activated macrophages. *Microbial Pathogenesis* **39**:3, 57-67. [CrossRef]
26. J REN, J PRESCOTT. 2004. The effect of mutation on virulence plasmid gene expression and mouse virulence. *Veterinary Microbiology* **103**:3-4, 219-230. [CrossRef]
27. Timo Jaeger, Heike Budde, Leopold Flohé, Ulrich Menge, Mahavir Singh, Madia Trujillo, Rafael Radi. 2004. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in Mycobacterium tuberculosis. *Archives of Biochemistry and Biophysics* **423**:1, 182-191. [CrossRef]
28. T Zahrt. 2003. Molecular mechanisms regulating persistent Mycobacterium tuberculosis infection. *Microbes and Infection* **5**:2, 159-167. [CrossRef]