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The Emerging Role of Gasotransmitters in the Pathogenesis of Tuberculosis

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Abstract

Mycobacterium tuberculosis (*Mtb*) is a facultative intracellular pathogen and the second largest contributor to global mortality caused by an infectious agent after HIV. In infected host cells, *Mtb* is faced with a harsh intracellular environment including hypoxia and the release of nitric oxide (NO) and carbon monoxide (CO) by immune cells. Hypoxia, NO and CO induce a state of *in vitro* dormancy where *Mtb* senses these gases via the DosS and DosT heme sensor kinase proteins, which in turn induce a set of ~47 genes, known as the *Mtb* Dos dormancy regulon. On the contrary, both iNOS and HO-1, which produce NO and CO, respectively, have been shown to be important against mycobacterial disease progression. In this review, we discuss the impact of O_2, NO and CO on *Mtb* physiology and in host responses to *Mtb* infection as well as the potential role of another major endogenous gas, hydrogen sulfide (H_2S), in *Mtb* pathogenesis.

Keywords

Mycobacterium tuberculosis; gasotransmitter; nitric oxide; carbon monoxide; hydrogen sulfide; heme oxygenase-1; reactive oxygen species; reactive nitrogen species; hypoxia

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1. Introduction

1.1. Global TB burden

The World Health Organization (WHO) has estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*), the bacterium that causes tuberculosis disease (TB). In 2014 ~1.5 million people died from TB, making *Mtb* the second deadliest pathogen of the 21st century after human immunodeficiency virus (HIV) [1]. HIV/AIDS exacerbates the global TB burden, as is evidenced by that fact that of the 9.6 million people newly infected in 2014, ~1.1 million (12%) were HIV positive [1]. Bacille Calmette-Guerin (BCG), the only vaccine against TB, is effective only in children and does not provide consistent protection in vaccinated adults. TB can be treated with existing drugs; however, the lengthy treatment regimen of 6–9 months often leads to patient noncompliance, which can result in the development of drug-resistant TB. Globally, an estimated 5% of active TB cases are multi-drug resistant TB (MDR-TB), defined as resistance to isoniazid and rifampicin, the two most potent first-line anti-TB drugs. In 2013, an estimated 480,000 people worldwide developed MDR-TB, of which an estimated 210,000 died [2]. Together with HIV co-infection, the emergence of MDR-TB represents a growing challenge to global public health that threatens to undermine the gains achieved through the last 80 years of medical advances. Clearly, it is of great importance to better understand the molecular mechanisms of *Mtb* pathogenesis in order to develop more effective drugs and therapies against this global threat.

1.2 Scope

In this review, we aim to provide a new perspective on the role of gasotransmitters in TB, specifically nitric oxide (NO), carbon monoxide (CO) and the newly emerging gas, hydrogen sulfide (H_2S). In addition, we will discuss the role of molecular oxygen (O_2), including hypoxia, as the concentration of O_2 plays a major role in TB disease progression. An emphasis is placed on the *in vivo* sources of these gases, and their roles in *Mtb* physiology and TB disease. This is followed by discussion of the mechanisms whereby *Mtb* senses and responds to these gases, including the Dos dormancy three-component system, the more recently described SenX-RegX two-component system, and iron-sulfur (Fe-S) cluster proteins. This review will not attempt in-depth descriptions of the chemical properties of these gases, but certain properties will be discussed where appropriate. For in-depth reviews on the chemical properties of these gases, we refer the reader to several excellent review articles [3–7]. This review will focus on the role of these host-derived intracellular gases in regulating *Mtb* disease progression with special focus on their specific roles in *Mtb* dormancy and in regulating host immune responses against TB.

1.3 Overview of Mtb physiology and disease dynamics

Mtb is a rod-shaped, acid-fast, obligate aerobe with a waxy lipid-rich cell wall and extremely slow growth rate (doubling time of ~18–20 hours in culture) [8]. Analysis of the *Mtb* genome has identified dedicated machinery for lipid biosynthesis, numerous virulence factors and sensor proteins that allow *Mtb* to respond to, and survive in, the hostile environment within host cells [9]. *Mtb* physiology is unique as studies have shown that *Mtb* can survive for up to 12 years in sealed tubes and remain fully virulent [10]. During

infection *Mtb* is exposed to a wide range of host substrates including organic acids, virtually all amino acids, nucleic acid precursors, nucleotides, lipids and numerous carbohydrates [11]. Thus, it is clear that *Mtb* must adjust its metabolism in response to the availability of environmental nutrients during the different stages of infection.

Mtb infection typically begins with the inhalation of *Mtb*-containing droplets aerosolized by an infected person [12]. The human lung has a total alveolar surface area of ~70 m^2, and an adult inhales and exhales ~11,500 liters of air each day [13]. Since the only route of *Mtb* transmission is by inhalation, the lungs are the primary site of infection. The first step of infection is the uptake of bacilli by alveolar macrophages (AM) by phagocytosis and the formation of phagosomes. Within the AM, fusion of phagosomes with acidic lysosomes is critical for bactericidal activity [12]. However, *Mtb* uniquely blocks phagolysosome fusion and acidification, thereby eliciting the rapid recruitment of neutrophils, inflammatory monocytes, interstitial macrophages and T-cells in the lungs followed by formation of cellular aggregates called granulomas. Containment of *Mtb* within granulomas results in an asymptomatic latent TB infection (LTBI), which is clinically defined by a reactive TB skin test without clinical symptoms of disease or radiological findings [14]. *Mtb* granulomas have been shown to be hypoxic in guinea pig and non-human primate (NHP) models and in a specific mouse model of TB [15, 16] (Fig. 1).

The occurrence of LTBI highlights a unique aspect of *Mtb* physiology that has been extensively studied, but is not fully understood; that is, the ability of *Mtb* to persist for years or decades in the host in a genetically controlled state of low metabolic activity referred to as dormancy [17]. As an obligate aerobe, *Mtb* requires oxygen for growth, and therefore, it is rational to conclude that the low oxygen environment of the TB granuloma contributes to this metabolic shutdown of the bacillus. Studies in the 1950s showed that open (oxygen rich) lesions from resected lung tissue from human TB patients yielded actively growing *Mtb* that were predominantly drug resistant. In contrast, *Mtb* cells isolated from closed (oxygen poor) lesions showed delayed growth, but were drug sensitive despite being refractory to antimycobacterial drug therapy [18, 19]. This pointed to O_2 as an important modulator of *Mtb* physiology and therapeutic intervention. Not surprisingly, a key objective in the TB field is to understand the mechanisms whereby *Mtb* persists in tissues for decades without replicating, to then abruptly resume growth and cause disease. Acquiring such knowledge will make a significant contribution towards new therapeutic interventions.

2. Role of oxygen in *Mtb* pathogenicity

2.1 Overview

Prior to the advent of TB drug therapy, patients were treated in sanatoria, where rest and fresh air were considered conducive to recovery [20]. The human host reaction to the bacillus was studied, and while many patients succumbed to the consumptive process, in other patients the bacilli were isolated and walled off by the body's defense mechanisms, leading to LTBI. One of the theories postulated was that the lower oxygen levels in these "walled off" areas inhibited the growth of the obligate aerobic mycobacterium, and the concept of "resting the lung" while the infection took its course became popularized. Various techniques to artificially collapse a lung to render a relatively hypoxemic

environment were then proposed, and “collapse therapy” marked the birth of thoracic surgery for the treatment of TB [21]. Methods of inducing “collapse therapy” included instilling air, liquid or spherical objects into the chest cavity to prevent the lung from completely expanding. By depriving the organism of oxygen, it was thought that the disease process would be curtailed. The advent of chemotherapy for TB marked the end of an era of collapse therapy for TB, and the attention of thoracic surgeons was then directed toward lung resection for TB (Fig. 2).

2.2 O_2 gradients during Mtb infection

It was believed that patients with pulmonary TB improved if they lived at altitudes above 2,000 m due to the lower oxygen tension [22]. Using radioisotope techniques, West and Dollery [22] measured regional perfusion and ventilation and calculated oxygen tension in the upright human lung. They observed that the partial pressure of alveolar oxygen (PaO_2) at sea level is approximately 132 mm Hg at the level of the first anterior interspace, decreasing to 89 mm Hg at the level of the fifth anterior [23]. These observations supported the popular belief that oxygen tension is a major determinant of the apical localization of pulmonary TB. However, an alternate explanation is that the effect of gravity on the erect human lung leads to a reduced pulmonary artery blood flow in the apical and subapical regions leading to higher O_2 tension [24, 25]. This reduced blood flow would impede tissue clearance and favor accumulation of *Mtb*, making the apical lobes ideal for *Mtb* growth and proliferation [26]. Although it can be argued that conventional *in vitro* tissue culture experiments expose *Mtb* to hyperoxic conditions, recent *in vitro* studies have shown that the intracellular environment of *Mtb*-infected macrophages *in vitro* may be hypoxic (< 10 mm Hg) [27]. The above discussion clearly highlights O_2 as a critical player in TB disease.

In the human TB lung, there is a gradient of oxygen tensions from the upper apices to the lower lobes. The flow rate of blood in the upper apex of the lung is reduced due to depressed pressure within the alveoli relative to pulmonary artery pressure in the upright position. Therefore, the apices have elevated oxygen tensions despite reduced ventilation due to restricted blood flow [28]. Several studies have shown that *Mtb*-infected lungs have various oxygen tensions, all of which are below the normal range seen in naïve subjects. This hypoxic environment within the *Mtb*-infected lung has important implications for drug distribution and bioavailability. The interplay between the host and *Mtb* requires in-depth analysis of various oxygen levels within the different compartments of human tuberculous lung, which could provide new insights into the underlying mechanisms of current anti-TB drugs activity. Despite recent progress, the physiology of *Mtb*-infected lung tissue is not yet fully resolved in terms of drug penetration and levels [29]. Animal models have been useful for shedding light on the dynamics of granuloma formation and TB disease progression. Granulomatous tissues in mouse models of TB have features that resemble those in humans despite some subtle differences [30]. Routine access to human tuberculous lung specimens for research purposes is unfortunately restricted by the paucity of these biopsies, and represents a major hindrance to TB research. This is important as there is no single animal model that accurately represents the full spectrum of human pulmonary TB. Another limitation of human TB lung studies is that the granuloma is dynamic in nature; hence real-

time experimentation is not possible in the human host. Mice, guinea pigs, rabbits, and non-human primates have the ability to form well defined granulomas upon *Mtb* infection.

2.3 Reactive oxygen species (ROS) and Mtb physiology

As a diffusible diatomic gas, O_2 plays important roles in host response to infection. As mentioned earlier, *Mtb* is exposed to low O_2 levels or hypoxic conditions within the lungs. Further, ROS and reactive nitrogen species (RNS) generated by host immune cells within the lung create a harsh intracellular environment against *Mtb*. The oxidative burst created by NADPH oxidase (NOX) of host phagocytic cells via catalysis of a single electron reduction of O_2 generates $O_2^{\bullet-}$ and further reduction of one more electron produces hydrogen peroxide (H_2O_2) by the activity of superoxide dismutase (SOD). Further, H_2O_2, in the presence of ferrous ions (Fe^{2+}) undergoes the Fenton reaction to produce hydroxyl radical ($OH^{\bullet}$). Overall, hypoxia and these free radicals constitute a strong host innate defense that can damage bacterial cell wall components, DNA, and proteins that protects the host against a wide range of bacterial infections including *Mtb* [31–34]. Mice deficient in the cytosolic p47(phox) gene, which is required for $O_2^{\bullet-}$ production, are susceptible to *Mtb* infection [35]. However, *Mtb* has evolved complex mechanisms that provide protection from the damaging effects of ROS and hypoxia. Components of the *Mtb* cell wall, including lipoarabinomannan (LAM), mycolic acids and phenolic glycolic lipids (PGL-1), serve as effective ROS scavengers [36]. Mycothiol and recently discovered ergothioneine act as redox buffers and protect *Mtb* from endogenous ROS [37, 38]. *Mtb* also possesses several ROS-scavenging gene products, which contribute to ROS detoxification and enhance *Mtb* survival. *Mtb katG* (catalase peroxidase) decomposes H_2O_2 into water and oxygen thereby protecting cells from the damaging effects of H_2O_2 [39]. *Mtb* Mg/Fe and Cu/Zn superoxide dismutases (*sodA* and *sod*C, respectively) also protect the cells from oxidative bursts and *Mtb* strains lacking these genes are susceptible to ROS-mediated killing in both macrophages *in vitro* and in the murine model of TB [40–42]. Other genes including alkyl peroxide reductase genes (*ahpC*/*D*/*E*) and lipoamide dehydrogenase (*lpd*) also contribute to *Mtb* survival against host-generated ROS [43]. In response to hypoxic conditions generated in the lung, the *Mtb* three-component heme sensor/regulator system (DosS/R/T) senses and binds to host O_2 thereby causing transcriptional adaptation that results in upregulation of *Mtb*-Dos dormancy regulon genes, which enables bacteria to persist in a non-replicative, low metabolic state [44] (see Section 6; *Mtb* gas sensors).

3. Nitric oxide

3.1 Overview

Since its initial characterization in the 1980s as a potent vasodilator followed by its recognition as ‘molecule of the year’ in 1992 [45], the gasotransmitter NO has been found to regulate a wide array of physiological functions such as neuromodulation, cellular signaling, maintenance of redox homeostasis and as an effector molecule during immune responses [46–49]. NO is produced endogenously by the nitric oxide synthase (NOS) enzyme family via NADPH- and O_2-dependent oxidation of L-arginine to L-citrulline and NO [50]. Three distinct mammalian NOS isoforms have been identified based on the location of their expression: NOS enzymes expressed by endothelial cells (eNOS, also referred to as NOS3)

and neurons (nNOS, also referred to as NOS1) are constitutively expressed and act distinctively in response to intracellular calcium levels in a calmodulin-dependent manner [51]. NO production for immunological functions is mediated by a calcium-independent inducible form, iNOS [51, 52]. iNOS is expressed predominantly in cells of myeloid lineage including macrophages and neutrophils and is generally induced by redox stress, exposure to microbial pathogens or bacterial endotoxins and pro-inflammatory cytokines such as IL-1, IFN-γ and TNFa (52). NO is a major RNS and reacts with atmospheric O_2, superoxide anions ($O_2^{\bullet -}$), heme/non-heme iron and thiol groups (−SH) of proteins to regulate a wide array of cellular signaling responses. One such response includes formation of peroxynitrite ($ONOO^-$) upon reaction with $O_2^{\bullet -}$. NO and other RNS including $ONOO^-$ can modify bacterial DNA, proteins, and lipids in host cells as well as in invading pathogens. Further, NO also interacts with iron-sulfur clusters or heme groups of proteins. Nitrite and nitrate, which were thought to be end products of NO metabolism, can be recycled to produce NO. Bacteria in the gut have also been shown to form nitric oxide under varying physiological concentrations of O_2 and L-arginine via acidification or reduction of nitrite [53–55].

3.2 Role of NO in host response to Mtb infection

Cells of the innate immune systems including macrophages and neutrophils are crucial for host defense against many bacterial infections including *Mtb*. One of their major anti-bacterial strategies includes upregulation of iNOS expression and production of NO [56–58]. Genetic knock-out studies with macrophages isolated from NADPH oxidase- or iNOS-deficient mice and in macrophages isolated from TB patients provided early evidence that ROS including $O_2^{\bullet -}$, and RNS including NO are important for host defense against *Mtb* [59, 60]. This was further substantiated by MacMicking, *et al.*, who demonstrated that iNOS-deficient mice are highly susceptible to *Mtb* infection and succumb to infection within ~45 days post infection [61]. Blocking iNOS function with inhibitors such as N6-(1-iminoethyl)-L-Lysine, aminoguanidine and NG-monomethyl-L-arginine resulted in rapid bacterial growth, increased disease pathology, high mortality and disease reactivation in murine TB models [62–64]. Similarly, inhibition of the iNOS-modulating cytokine TNFa also leads to *Mtb* reactivation in a murine model of latent TB [65]. A more recent study has shown that NO regulates TB immunopathology and tissue damage by regulating the levels of pro-inflammatory cytokine interleukin1β (IL1β) in both IFNγ-dependent and independent manners [66]. Compared to murine models, the protective role of NO in *Mtb*-infected humans is poorly understood. Nonetheless, immunohistochemical analysis of human TB lung tissue and AM revealed the presence of iNOS, NO, nitrotyrosine and increased exhaled NO in TB patients, suggesting a role for NO in human TB [67–69]. Further, AM from LTBI patients may kill *Mtb* in an iNOS-dependent manner [70, 71]. Similarly, Rich *et al.* reported that inhibition of intracellular *Mtb* growth correlated with elevated levels of NO and iNOS in AM following *Mtb* infection [72]. Kuo *et, al.* showed that increased NO generation by AM of TB patients plays an autoregulatory role in amplifying the synthesis of proinflammatory cytokines [73]. In the human promyelocytic cell line HL-60, vitamin D3 treatment resulted in significant increase in NO production and enhanced inhibition of *Mtb* growth [74]. NO is also thought to be important for the formation of granulomas thereby protecting the host from *Mtb* dissemination [75]. Interestingly, when peripheral blood monocytes obtained from patients with active TB where infected with *Mtb in vitro*, increases in both NO and TNF-α

levels were observed. However, the same cells obtained from patients with MDR-TB produced little NO and TNF-α following *Mtb* infection, suggesting a role for NO in TB-associated immunosuppression and TB drug resistance [76]. Overall these studies clearly suggest that NO plays significant role in host response against *Mtb*.

3.3 Role of NO in Mtb physiology

As discussed above, iNOS/NO is an important component of the host defense strategy against *Mtb* infection; however, *Mtb* has developed a number of complex mechanisms to resist, subvert and counter the damaging effects of intracellular NO and other RNS. Darwin *et al.* have shown that the *Mtb* proteasome is crucial for bacterial resistance against host-generated oxidative and nitrosative stress, likely through the degradation of stress-damaged proteins [77]. Other studies showed that the *noxR1* and *noxR3* genes of *Mtb* confer protection against NO, RNS and ROS [78, 79] Further, *Mtb ahpc* was protective during RNS-induced necrosis, apoptosis and also protected the bacterium from DNA damage by detoxifying $ONOO^-$ [80]. Interestingly, transcriptomic analysis of iNOS-deficient mice revealed that *Mtb* faces significant oxidative and nitrosative stress within the phagosomal environment. However, under these conditions *Mtb* undergoes drastic transcriptional adaptation which enables to switch from aerobic to anaerobic respiration and entering dormancy [81]. Further, during hypoxia, nitrate is reduced by *Mtb* to nitrite which also assists in switching to anaerobic respiration and inducing dormancy [82]. Similarly, nitrate respiration was also important for *Mtb* survival under acidic and nitrosative stress environments [83]. Voskuil *et al.* showed that non-toxic concentrations of NO inhibit *Mtb* respiration and growth, and result in the induction of *Mtb* dormancy regulon genes [84]. Similar to O_2, NO is also sensed by the *Mtb* three-component system DosS/R/T, which causes *Mtb* to enter dormancy *in vitro* [44, 85, 86]. Interestingly, Cunningham-Bussel *et al.* have shown that *Mtb* also produces significant levels of nitrite during infection of human macrophages, which assists its survival under hypoxic conditions by modulating bacterial respiration, ATP consumption and transcriptional modulation of stress associated genes [87]. Overall, these studies show that while NO is important for host defense, *Mtb* has evolved potent NO-mitigating machinery (Fig. 3).

4. Carbon monoxide (CO)

4.1 Overview

While initially regarded as a toxic environmental gas, CO is now known to be an enzymatic by-product of the heme oxygenase (HO) system and a key intracellular messenger that regulates numerous host physiological responses [5]. HO enzymes convert free heme, sourced primarily from hemoglobin released from erythrocytes, into CO, free iron (Fe^{2+}) and biliverdin in equimolar ratios [88]. Two major HO enzymes have been characterized in mammals: the inducible form, HO-1, and a constitutive isoform, HO-2. While HO-2 is constitutively expressed almost exclusively in the brain, HO-1 is ubiquitously expressed at very low levels in almost all cell types, but its expression is highly induced by cellular redox stress, hypoxia, bacterial lipopolysaccharides and many pathogenic challenges including mycobacterial species [5, 89]. The HO system produces ~86% of endogenous CO; the remainder is contributed by lipid peroxidation, bacteria, photo-oxidation, and xenobiotic

metabolism [5]. Verma *et al*. provided the first evidence that CO has a physiological role and, like NO, acts as a signaling molecule to regulate cGMP production [90]. Additional studies showed CO to be a key regulator of vascular and cardiac functions [91], inflammation [92, 93] and apoptosis [94, 95]. Later studies revealed cytoprotective roles for CO, which eventually lead to evaluation of CO as a therapeutic intervention in many pathophysiological conditions [96, 97].

4.2 Role of CO in bacterial pathogenesis

While the functions of CO in host responses to bacterial infections have been studied, demonstration of a direct role for CO in bacterial physiology has so far been limited to its bactericidal activity in *E. coli*, *P. aeruginosa*, and *S. aureus*, which results in death of the bacillus [98, 99]. Roles for CO in bacterial pathogenesis have been shown in models of bacterial disease, including sepsis and malaria [100]. Administration of a carbon monoxide releasing molecule (CO-RM) resulted in enhanced bacterial clearance and improved outcomes in a HO-1-deficient mouse model of severe sepsis [101]. CO has also been shown to enhance the rate of macrophage phagocytosis of *E. coli* through upregulation of TLR-4 expression via p38 MAPK signaling [102]. A similar study reported that macrophage-specific deletion of HO-1 increases macrophage death upon infection with *E. coli* and *E. faecalis* and that this effect was reversed by administration of exogenous CO [103]. In malarial disease caused by the parasite *Plasmodium*, Pamplona *et al*., have shown that pharmacological induction of HO-1 or CO prevents experimental cerebral malaria (ECM) in mice by inhibiting the release of free heme. This prevents disruption of the blood brain barrier, and regulates CD8 T-cell responses [104]. Similarly, Jeney *et al*. demonstrated that the HO/CO system, along with NO, provides disease tolerance during cerebral malaria via suppression of T-cell responses [105]. Taken together, these studies show that host-derived CO plays an important role in host defense and bacterial physiology.

4.3 Role of CO in Mtb physiology and pathogenicity

One anti-bacterial strategy of host phagocytes is the upregulation of HO-1 and the production of CO via HO-1 enzymatic activity. However, unlike other bacterial pathogens, *Mtb* tolerates physiological levels of CO due to its expression of a CO-resistance gene, *cor* [106]. The first major advancement in our understanding of a role for CO in *Mtb* physiology came from studies showing that like O_2 and NO, CO can independently bind to *Mtb* heme sensor kinase proteins DosS and DosT as confirmed by the formation of heme-carbonyl species upon exposure to CO [44]. Further, exposure of *Mtb* cells to CO-RM-derived CO or CO gas results in increased expression of *dosR*, *hspX* and *fdxA* genes of the *Mtb* dormancy regulon [44]. *In vitro* infection studies using macrophages isolated from wild-type and HO-$1^{-/-}$ mice showed that physiological levels of host-generated CO induce a transcriptional profile of *Mtb* dormancy-inducing genes comparable to that of NO. This transcriptional regulation is specific to CO and not the other products of HO-1 enzymatic activity [107, 108]. These studies also demonstrated that while induction of the dormancy regulon genes (discussed later) by NO requires the activity of both heme sensor kinases DosS and DosT, the binding of CO to DosS alone is sufficient to induce the *Mtb* dormancy regulon. These data demonstrate a direct role for CO in initiation of *Mtb* dormancy, and that

HO-1-derived CO and iNOS-derived NO may act in synchrony or independently to determine the outcome of infection.

We and others have shown showed that HO-1 levels are significantly increased in macrophages *in vitro* and in the lungs of mice following *Mtb* infection [107, 108]. In the infected lung, HO-1 is upregulated in immune cells that surround granulomas, suggesting that increased production of CO may be a host strategy to induce dormancy and restrict bacterial dissemination [108]. While these studies highlight the role of HO-1-derived CO in *Mtb* dormancy, the role of CO in active TB disease is still unclear. Nonetheless, during non-tuberculous infections (NTM) caused by *Mycobacterium avium* (*M. avium*), HO-1 confers host protection by suppressing the expression of monocyte chemoattractant protein-1 (MCP-1) and chemokine receptor 2 (CCR2) resulting in the formation of compact granulomas, thereby reducing bacterial dissemination. HO-1 also counteracts the cytotoxic effects of heme and inhibits apoptosis of infected macrophages [109, 110]. While based on a HO-1-deficient mouse model of *M. avium* infection, these studies did not demonstrate a direct role for CO, a technically challenging task. However, since CO is known to have anti-apoptotic, anti-inflammatory and cytoprotective functions in macrophages when challenged with bacterial lipopolysaccharide (LPS), it is reasonable to postulate that the cytoprotective activity of HO-1 during *M. avium* infection is attributable to CO [111]. Further, these studies point to HO-1 and CO as a potential targets for therapeutic interventions against *M. avium* infection.

HO-1 and HO-1-derived CO play an important role in host anti-inflammatory and anti-oxidative responses during various pathological conditions [92, 97]. Notably, active TB is characterized by dysregulated inflammatory responses such as heightened immune cell infiltration, increased levels of pro-inflammatory cytokines and hemorrhagic inflammation resulting in hemoptysis and extensive lung tissue damage [112–115]. Andrade *et al.* showed that HO-1 levels are significantly elevated in the serum of patients with active pulmonary TB compared to patients with LTBI, and suggested that elevated HO-1 levels could be a potential TB biomarker [116]. A later study by the same group showed that increases in HO-1 levels in TB patients corresponded with decreased expression of matrix metalloproteinase-1 (MMP-1), which drives immunopathology during TB [117, 118]. This inversely proportional expression of HO-1 and MMP-1 was specific to TB and was not observed in other lung diseases. The authors also demonstrated that CO specifically reduces the expression of MMP-1 via suppression of c-Jun/AP-1 activation and suggested a potential therapeutic role for CO in host protection against TB disease progression.

The dysregulated immune responses and increased influx of immune cells to the infection site during active TB results in substantial oxidative and nitrosative stresses that likely result in extensive tissue damage. In contrast, dormant *Mtb* within granuloma remain non-pathogenic and drug insensitive, leading to LTBI. These two disease phenotypes in TB are strikingly similar to malaria, wherein a clinically latent pre-erythrocytic stage is required to establish infection, and a subsequent erythrocytic stage is responsible for active malarial disease [119]. Since application of HO-1 and CO has been shown to protect mice from malarial disease pathology, a similar therapeutic strategy for *Mtb* infection may have merit.

Overall, while the role of CO in TB is not fully understood, it is clear that it plays a key role in both controlling mycobacterial dissemination and inducing dormancy *in vitro*. Further, as HO-1/CO are important for host defense against oxidative and inflammatory damage, their therapeutic application at the site of infection may play a significant role in minimizing exacerbated inflammation and oxidative damage during TB (Fig. 4).

5. Hydrogen Sulfide (H_2S)

5.1 Overview

H_2S has recently emerged as the fourth physiologically important gas that mediates multiple processes in mammalian systems including hypertension, atherosclerosis, heart failure, diabetes, inflammation, neurodegenerative diseases and asthma [120–126]. H_2S can be produced in the human body by resident microbes [127] or by dedicated enzymatic machinery consisting of three independent enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase β-MST) along with cysteine aminotransferase (CAT) [128] (Fig. 5). The overall chemical properties of sulfide (defined here as $H_2S + HS^-$) relevant to biological systems are briefly outlined below; further details can be found in recent reviews on the biochemical properties of sulfide [6, 129–131]. H_2S is a weak acid, with a pK_a of *ca.* 7 for the dissociation $H_2S \leftrightarrow H^+ + HS^-$, the second pK_a is high enough that the sulfide anion (S^{2-}) almost certainly has no biological relevance. This means approximately 28% of sulfide is present as the protonated nonelectrolyte species (H_2S, hydrogen sulfide) at pH 7.4. As an uncharged small lipophilic molecule, the fact that protonation/deprotonation is extremely rapid means that membranes are quite permeable and so sulfide produced in one compartment will rapidly distribute within the biological milieu. The volatility of H_2S also demands relatively rapid exit to a headspace, requiring careful attention to experimental configuration. The chemical reactivity of sulfide is dominated by the fact that, like thiol, it is a strong nucleophile, much more so in the hydrosulfide anion form (HS-) than H_2S. This means that HS- is the species that exerts the majority of (but not all) biochemical reactivity. The nucleophilicity of sulfide is reflected in its electronegativity, second only to oxygen (except for fluorine). Unlike oxygen, sulfur is able to utilize its *3d* orbitals for an expanded valence shell thereby allowing multiple bonds and formal oxidation states ranging from −2 (*e.g.* H_2S) to +6 (*e.g.* SO_4^{2-}).

Being electrophiles, transition metal ions react with sulfide by both complete (oxidation-reduction) and partial (bonding) electron transfer. The product of oxidation-reduction is the reduced metal and elemental sulfur (So). Elemental sulfur exists as a polymer, the most common configuration being S_8. In the case of bonding, the metal-sulfide bond is a special type of bond, called a coordinate bond. The toxicity of sulfide in humans is dominated by its reactions with metals, specifically, with mitochondrial cytochrome oxidase at the site of O_2 binding (the binuclear Cu_B/heme a_3) and with hemoglobin which, through the intermediacy of a high valency heme-oxygen complex, produces sulfhemoglobin, which contains a covalent heme modification. Sulfide anion is also a key component of a large class of iron proteins containing iron-sulfur centers, which serves both oxidation/reduction and signaling roles. Undoubtedly, the best studied biological system of metal-sulfide interaction is in a symbiotic mollusk/bacterial symbiosis, where cytoplasmic hemoglobin in the host delivers

both O_2 and sulfide to the bacterium for metabolism [132]. The properties of the mollusk hemoglobin are elegantly tuned to carry these two molecules, involving configuration of the heme pocket and proximal and distal protein residues.

Partially reduced oxygen species ($O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$) are also electrophiles and react with sulfide. The rates of reaction, however, are highly variable, and in the *in vivo* environment these reactions are not likely to be important under most conditions due to the low concentrations of both these species and also sulfide. Finally, sulfide also reacts with other electrophilic species, of particular interest being the newly discovered species 8-nitro-cyclic GMP [133]. There is evidence that this reaction plays a key role in the signaling properties of this molecule. Sulfide also reacts with oxidized sulfur-containing species, for example, disulfide RSSR'. The product is thiol and persulfide: $HS^- + RSSR' \rightarrow RSS^- + R'S^-$ (contrary to some statements in the literature, sulfide does not react with thiol; in general, nucleophiles do not react with other nucleophiles). Similar further reactions also produce polysulfur species $R(S)_nH$, in great abundance. This is true for both small thiols (*e.g.* glutathione) and also protein cysteine thiol. Indeed, it may well be that these species are more important functionally than sulfide itself. In spite of the lack of relevant data, there are observations about the role of H_2S in mammals and other prokaryotes [127, 134, 135].

5.2 A role for H_2S in TB?

It is not known whether H_2S impacts the outcome of *Mtb* infection and TB disease in humans. Regardless, the physicochemical properties of H_2S, as well as its established role in modulating immunity point to a potentially important role for this gas in TB. Both CBS and CSE have been found critical for proper alveolarization and functioning of the lung [136]. Since the human lung is the primary organ of *Mtb* infection in which the bacillus can reside for decades, it is reasonable to posit that *Mtb* may have mechanisms for sensing and responding to H_2S. Also, since CBS and CSE play essential roles in human health [136–138], it is plausible that H_2S via its immunomodulatory function is necessary for protection against *Mtb* infection. Studies on the role of H_2S on *Mtb* pathogenesis are eagerly awaited.

However, there are observations on the role of H_2S in mammals and other prokaryotes that can be extrapolated to comment on the possible role that H_2S may play in TB disease. For example, exposing mice to low amounts of H_2S induces a reversible hibernation-like state characterized by lowering of body temperature, and reduced energy expenditure in the form of reduced ATP consumption and low metabolic rate (as evaluated by substantially decreased CO_2 production and O_2 consumption) [139]. Induction of this hypo-metabolic state by H_2S, referred to as suspended animation, is an oxygen-dependent phenomenon [140]. It is believed that the inhibition of O_2 utilization and induction of suspended animation in low O_2 conditions could have a host-protective role [140]. There are parallels that can be drawn from this H_2S-induced suspended animation state and the characteristic features associated with dormancy in *Mtb*. For example, during dormancy *Mtb* is exposed to severe environmental stresses (*e.g.*, low pH, O_2 and carbon source limitation) that induce a metabolic state characterized by decreased respiration, low ATP levels, reduced growth, low O_2 consumption and reduced metabolic rates.

H_2S primarily affects mitochondrial respiration by targeting complex IV of the respiratory chain, i.e., cytochrome c oxidase [141]. Based on historical studies using manometry [142] and in a more recent drug study [143], *Mtb* is known to exhibit immense respiratory flexibility, which is a key requirement for maintaining an intracellular pathogenic lifestyle. It possesses two proton-pumping complexes: NADH dehydrogenase I, which is dispensable for growth, and an aa3-type cytochrome c oxidase, which provides the means of O_2 reduction [144]. More importantly, *Mtb* also possess an alternate terminal oxidase, cytochrome bd oxidase, a high-affinity alternate terminal oxidase that can partially compensate for the loss in activity of the bc1-aa3 complex [144]. Therefore, it will be of significant interest to determine whether H_2S affects *Mtb* respiration and consequently *in vivo* pathogenesis.

Lastly, there is a growing appreciation for the complex interactions between different intracellular gases such as NO, CO and H_2S as they can all interact with the host (and potentially *Mtb*) electron transport chain [145–150]. Furthermore, NO and CO can also regulate H_2S levels by targeting the CBS heme prosthetic group [151–153].

6. *Mtb* gas sensors

6.1 The Mtb Dos dormancy regulon

The success of *Mtb* as a pathogen is based in part on its ability to enter into dormancy, a genetically and physiologically controlled non-replicative state characterized by metabolic quiescence. In this reversible state, *Mtb* can survive the harsh intracellular environment inside the host [154]. Of note, dormancy in *Mtb* is a physiological state of persistence and should not be confused with TB latency or LTBI, which are clinical descriptors indicating the absence of TB disease symptoms in infected individuals. The transition into dormancy occurs via marked changes in gene expression, including up-regulation of the widely studied 47-gene *Dos* regulon in response to CO, NO or hypoxia [155, 156]. In addition to the genes of the *Dos* regulon, other gene sets, such as the enduring hypoxia response (EHR) genes, may play a role in dormancy; however molecular mechanisms of their regulation are largely unknown [157, 158]. Transcriptional analysis under hypoxic conditions *in vitro* revealed that even though ribosomal and metabolism genes are downregulated under low-O_2 conditions, the Dos regulon genes are significantly upregulated [159]. Similar to the response to hypoxia or low O_2 levels, other host-generated stresses such as CO and NO also upregulate these dormancy regulon genes. While many genes of this regulon are still being characterized, they are predicted to be involved in modulating *Mtb* physiology. These include genes with chaperone functions (*acr* or Rv2031c), ferredoxin modulation (*fdxA* or Rv2007c), nitrate/nitrite transporter (*nark2* or Rv 1737), ribonuclease reductase (*nrdZ* or Rv0570) and triglyceride synthase (*tgs1* or Rv3130) [19, 160].

The genes of the dormancy regulon are upregulated when *Mtb* senses host-generated hypoxia, NO and CO via the heme-containing kinases DosS and DosT, which along with a single response regulator, DosR, comprise the DosR/S/T three-component system [44, 107, 161]. As discussed earlier, our studies and others have shown that the redox or ligation status of the heme iron in DosS and DosT modulates binding to O_2, NO and CO either together or independently. This shows that these host-generated intracellular gases are central to *Mtb*

disease progression [19, 44]. This also emphasizes the fact that the success of *Mtb* as a pathogen can be largely attributed to its unique ability to sense and respond to NO, CO or low O_2. Several lines of evidence point to these diatomic gases as modulators of dormancy. Firstly, *Mtb* is an obligate aerobe that requires oxygen as a terminal electron acceptor. However, the uncanny ability of *Mtb* to survive for more than a decade in test tubes without oxygen (or at least extremely low concentrations of oxygen) points to a novel aspect of *Mtb* adaptation to low oxygen environments. Secondly, it has been conclusively demonstrated that iNOS, and therefore NO is crucial for protection of mice against *Mtb* [61]. Further studies in patients with active pulmonary TB confirm the importance of NO in *Mtb* pathogenesis [59, 60, 35–76, 162]. Unfortunately, despite several elegant *in vitro* studies [107, 108, 163] a clearly established role for either HO-1 or CO in *Mtb* pathogenesis has yet to be demonstrated and represents an unexplored area of investigation.

It should be noted that the lack of oxygen and the presence of sufficient levels of NO and/or CO inhibit *Mtb* respiration and ultimately retard growth. An important difference between NO and CO is that CO can only react with ferrous (Fe^{2+}) iron whereas NO reacts with Fe^{2+} and Fe^{3+}. This raises the obvious question as to how concentration gradients of oxygen, NO and CO, as well as the different affinities of DosS and DosT for these gases contribute to *Mtb* disease.

Most studies showing the effect of gases on *Mtb* have been performed *in vitro* or in mouse models of TB and their relevance to human TB is yet to be established. However, recent studies in our laboratory using tissue samples from freshly resected human tuberculous lungs have clearly shown that significant levels of ROS/RNS are indeed present in human TB lungs. Interestingly ROS/RNS levels were directly proportional to the extent of tissue damage, suggesting a gradual disease progression.

6.2 Mtb iron-sulfur (Fe-S) cluster proteins

Proteins that contain iron-sulfur cluster [Fe-S] prosthetic groups are found in virtually all organisms and provide a mechanism for cells to sense changes in redox and respond to intracellular gases. The *Mtb* WhiB family of proteins WhiB1 (Rv3219), WhiB2 (Rv3260c), WhiB3 (Rv3416), WhiB4 (Rv3681c), WhiB5 (Rv0022c), WhiB6 (Rv3862c) and WhiB7 (Rv3197A)] contain four cysteine residues arranged in a Cys–X-$_{14–22}$-Cys-X_2-Cys-X_5-Cys motif, with the exception of WhiB5 [164]. Studies of homologs in *Streptomyces spp* suggested that these proteins are Fe-S clusters proteins [164]. Indeed, *Mtb* WhiB3 is a 4Fe-4S cluster protein that maintains intracellular redox balance and responds to oxygen and NO [165]. Recently, WhiB3 was shown to regulate the production of a redox buffer, ergothioneine, which is critical for *Mtb* virulence and drug susceptibility [38]. The WhiB proteins likely acts as redox sensors via their Fe-S clusters and are critical in maintaining redox homeostasis, a key determinant of *Mtb* virulence [19, 38]. The seven members of the *Mtb* WhiB family perform diverse functions including cell division (WhiB2) [166], fatty acid metabolism and pathogenesis (WhiB3) [165, 167, 168], oxidative stress (WhiB6) [169, 170] and antibiotic resistance (WhiB7) despite only moderate sequence homology among themselves [171, 172].

Since the discovery of a WhiB-like protein in *Streptomyces spp* in 1992, a long-standing question has been whether these proteins are DNA-binding proteins that regulate gene expression [173]. Nearly 20 years later, it was shown that *Mtb* WhiB3 binds DNA [168]. Important features of its DNA binding capacity, which was extrapolated to other WhiB members, was that oxidized apo-WhiB3 binds DNA much stronger than either holo-WhiB3 or reduced apo-WhiB3. Subsequently, it was shown that *Mtb* apo-WhiB1, apo-WhiB2 and apo-WHiBTM4 bind DNA strongly in contrast to the holo-forms, which bind DNA weakly or not at all [164]. These findings revealed that DNA binding and transcriptional activity are modified by environmental conditions such as hypoxia, free radicals such as NO and oxidative stressors that influence the redox state of the 4Fe-4S cluster (of the holo-form) or the oxidation states of the Cys residues (of the apo-form). Overall, it appears that *Mtb* has evolved a sophisticated family of proteins that function as a flexible redox switch in response to oxido-reductive stress that causes an imbalance in intracellular redox homeostasis.

Redox homeostasis in mycobacteria is distinct from other intracellular pathogens as they lack a conventional glutathione system and homologues of the prototype sensor proteins in other intracellular pathogens such as SoxR, ArcAB, FNR and OxyR (a pseudogene in *Mtb*) [172–174]. Instead, *Mtb* uses two distinct redox couples, mycothiol and ergothioneine, and harbors at least 50 proteins containing Fe–S clusters that facilitate an intercellular life cycle [37, 175]. Interestingly, *Mtb* has a higher incidence of Fe–S cluster proteins (~6.5 motifs/1000 ORFs) than other aerobic bacteria (2.8 motifs/1000 ORFs), underscoring the importance of Fe–S cluster proteins to pathogenic adaptations of *Mtb* [176, 177].

6.3 Other gas-sensing systems

Apart from DosS/R/T, *Mtb* has other heme sensor kinase and response regulator protein systems that are predicted to play roles in its metabolic adaptation, including the SenX3-RegX3 system and the PhoPR systems [178, 179]. Transcriptomic analysis of a *senX3-regX3* knockout strain of *Mtb* showed downregulation of several DNA repair and protein synthesis genes as well as genes important for survival under hypoxic conditions such as *cydB* and *gltA1* [180]. Further, this *Mtb* knockout strain fails to establish successful infection of macrophages *in vitro* and in guinea pig and murine models of TB [181–183]. More recently, SenX3 was shown to sense and bind O_2, NO and CO. Binding of O_2 to SenX3 is associated with growth stimulation during reactivation whereas binding of NO and CO to SenX3 inhibits growth via inhibition of kinase activity [184].

7. Future challenges and conclusions

The development of highly effective therapeutic interventions such as new antimycobacterial drugs and vaccines hinges on understanding the mechanisms by which *Mtb* can persist for decades in the human lung to ultimately cause disease. Given the ubiquitous nature of O_2, NO, CO and H_2S, it is not surprising that some of these endogenous gases play critical roles in a wide range of pathophysiological processes. The importance of these gases in human health is underlined by genetic deficiency and polymorphism studies. For example, the pathophysiological consequences of deficiencies in HO-1 (systemic inflammation,

intravascular hemolysis, asplenia, vulnerability to stressful injury) [185, 186], CBS, CSE or 3-MST (hyper-homocysteinemia, mercaptolactate-cysteine disulfiduria, mental retardation and aggressive vascular disease, neuoroblastoma and hepatoblastoma) [187–189] and iNOS (susceptibility to TB, and a wide range of diseases) [190] have profound effects on human health. Therefore, dysregulation of the levels of these gases at the site of infection could lead to ineffective drug treatment or impair inflammatory responses to exacerbate disease.

A practical advantage in studying the role of NO, CO and H_2S in *Mtb* infection is that mice with deficiencies in expression of iNOS, HO-1, CBS, CSE and 3-MST are available for experimentation. To confirm the clinical relevance of these gases, findings from these studies should be expanded to the analysis of human pulmonary TB tissue samples. Unfortunately, a major impediment to these studies is the paucity of freshly resected human TB lung tissue. Of particular interest would be to determine what cell populations in animal and human TB lung tissues express CBS, CSE, 3-MST, iNOS and HO-1, and their respective levels. It is important to note that in the case of iNOS and HO-1, which are expressed at the site of infection [67, 107, 108], gradients of NO, CO and O_2 are likely to exist in hypoxic *Mtb* granulomas [19, 191]. This presents an interesting opportunity for studying crosstalk among the gases since CBS and iNOS contain heme moieties, which represent potential interaction sites for NO, CO and O_2.

Another important albeit unexplored area of investigation is the role of these gases in antimycobacterial drug susceptibility *in vivo*. First considered for NO, it is tempting to speculate that gasotransmitters play a role in antimycobacterial drug efficacy [67]. On the other hand, since these gasotransmitters are ubiquitous, pharmacologic modulation of gene expression or the application of iNOS, HO-1, CBS, CSE or 3-MST end products has long been proposed as a therapeutic avenue based on model studies [3–7]. Understanding how *Mtb* directly interacts with these gases, their corresponding targets, how these targets discriminate between these gases, and the signaling responses induced by these gases will be of substantial value. NO, CO and O_2 have been shown to target the heme irons of *Mtb* DosS and DosT. However, targets other than the WhiB family proteins and the SenX3-RegX3 two-component system also exist. Further, a detailed understanding of how these gasotransmitters target the *Mtb* electron transport chain, which contains numerous heme and Fe-S cluster prosthetic groups, should provide new information on energy metabolism, and how the bacillus is capable of surviving under harsh intracellular conditions including hypoxia and gradients of NO, CO and H_2S. The *Mtb* electron transport chain is remarkably plastic and capable of rapidly re-routing electrons around specific inhibition by switching between terminal oxidases. Despite the many potential targets, very little is known about this ATP-generating pathway in *Mtb*. Many provocative questions can emerge from such studies. For example, do these gasotransmitters inhibits or stimulate respiration, and do they have a predominantly bactericidal or bacteriostatic effect on *Mtb*?

In conclusion, it is anticipated that as more is learned about how these gasotransmitters regulate host immunity and directly targets *Mtb*, many new avenues of research will open up, compelling researchers to reassess existing paradigms for *Mtb* persistence, and to seek new testable hypothesis.

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Highlights

- One-third of the world's population is infected with *Mtb*, the bacterium that causes TB.
- Phagocytosis, ROS and RNS are important host responses to restrict initial *Mtb* infection.
- iNOS and HO-1 enzymatic activity is important for host anti-mycobacterial responses.
- *Mtb* heme sensor kinases bind to O_2, NO and CO to modulate dormancy and reactivation.
- Interaction of O_2 and NO with *Mtb* 4Fe-4S cluster proteins regulates redox homeostasis and metabolism.

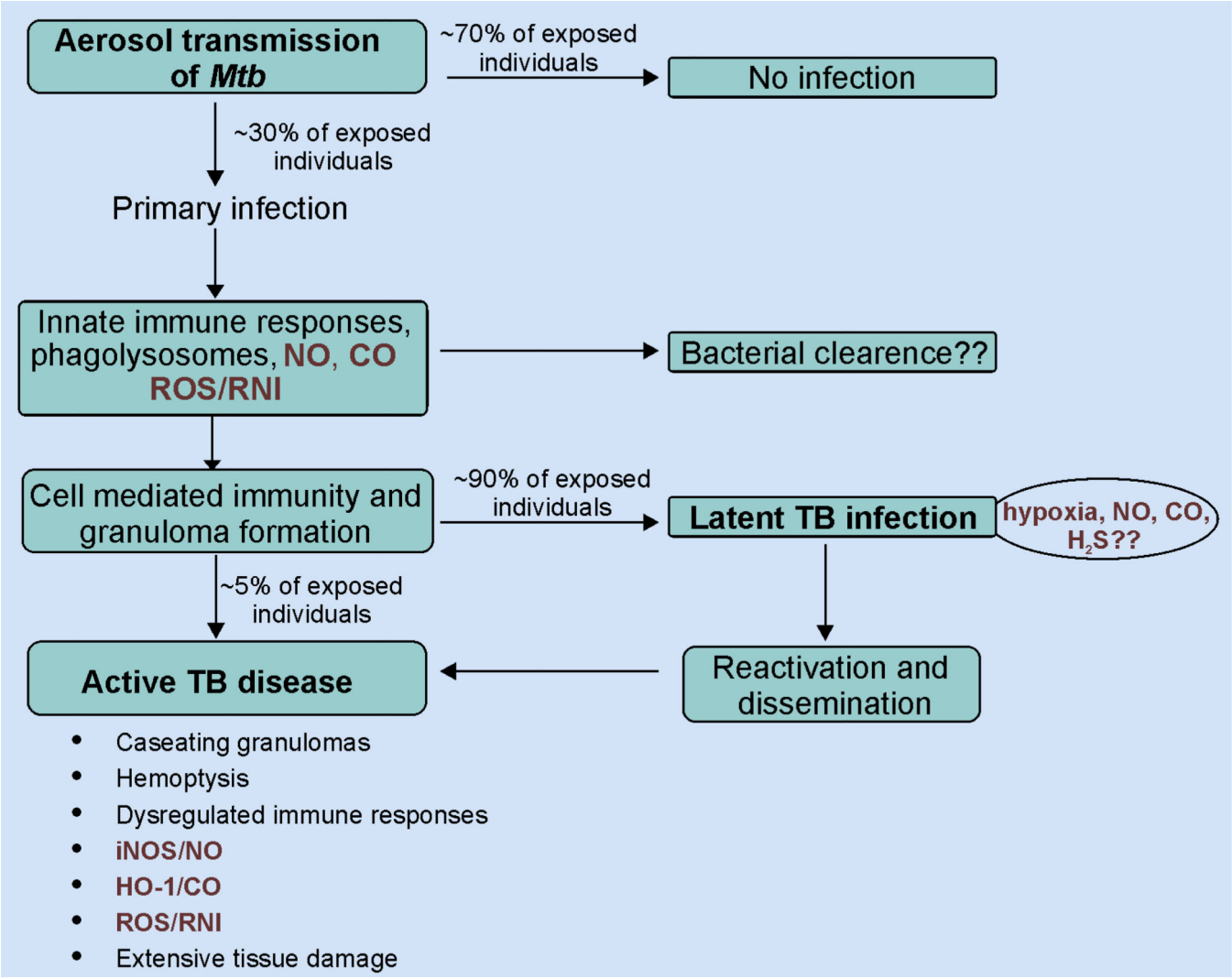

Figure 1. Schematic overview of *Mtb* infection

Mtb infection is initiated when an individual is exposed to aerosolized bacteria. Of those exposed, ~70% remain uninfected, whereas ~30% develop primary infection. The host immune response to the primary infection is initiated by innate immune responses of macrophages and neutrophils via phagocytosis, upregulation of iNOS and HO-1 and production of ROS/RNS. This is followed antigen presentation by dendritic cells, cell-mediated immunity and further infiltration of immune cells. These responses initially restrict bacterial replication and dissemination and lead to the formation of granulomas. In most cases, *Mtb* resides within the granuloma in a non-replicating dormant state leading to LTBI. Approximately 90% of individuals with LTBI remain asymptomatic while ~5% develop active TB disease, characterized by massive hemoptysis, dysregulated immune responses and extensive tissue damage.

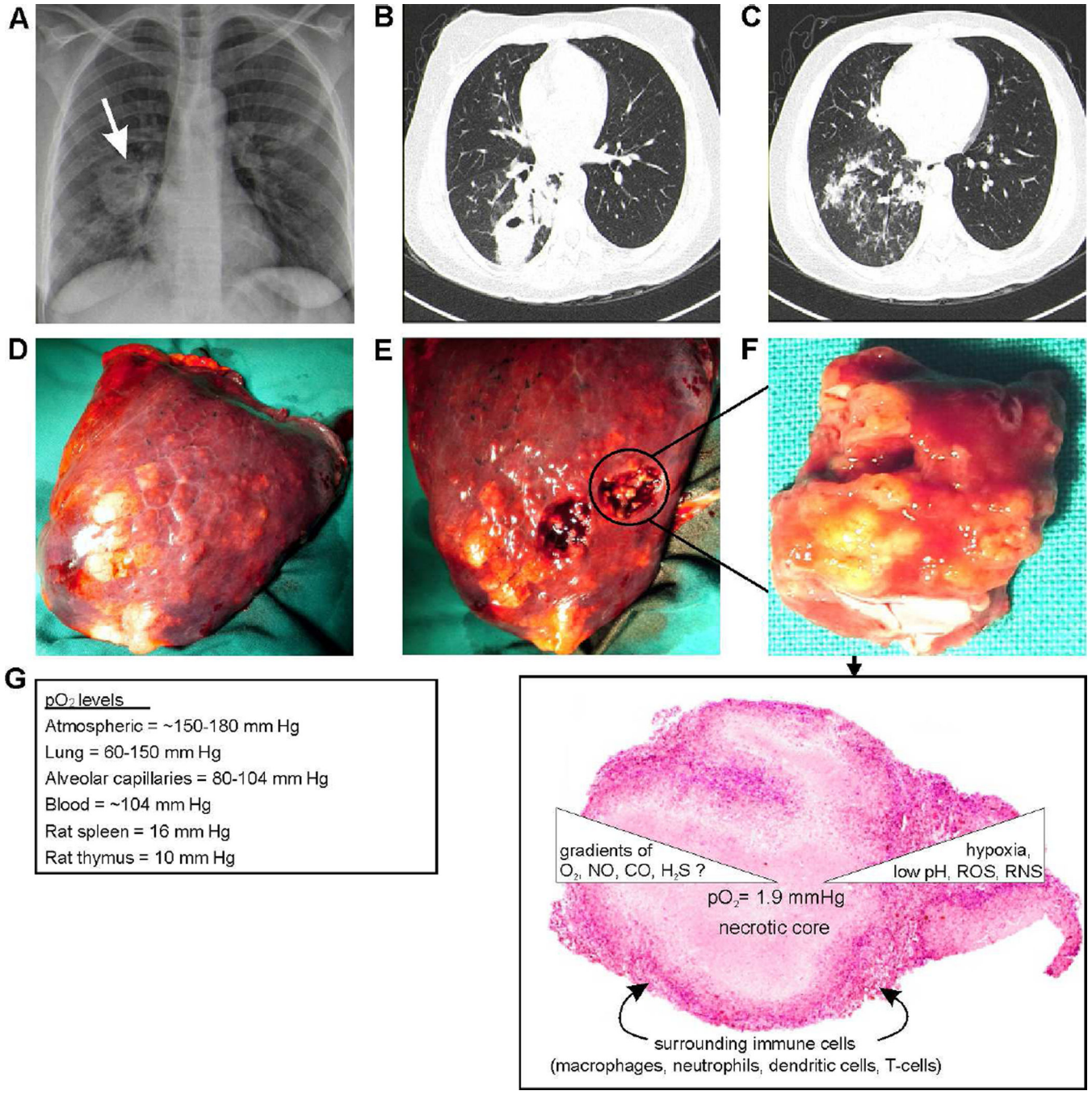

Figure 2. Typical chest radio graph, HRCT and granuloma of a TB patient

(A) Plain chest radiograph of a 46 year old woman with active pulmonary tuberculosis, demonstrating a thick walled cavity in the right lung with associated parenchymal opacification. (B, C) High resolution computed tomography (HRCT) images illustrate the location of the cavity in the apical segment of the right lower lobe with the associated tree-in-bud appearance in the basal segments that are characteristic of active tuberculosis. (D, E, F) Right lower lobectomy specimen with macroscopic features of active pulmonary tuberculous nodules with central caseous necrosis that is evident after sectioning. (G)

Representative tuberculous granuloma with central necrotic core surrounded by immune cells which create a gradient of O_2, NO, CO and possibly H_2S.

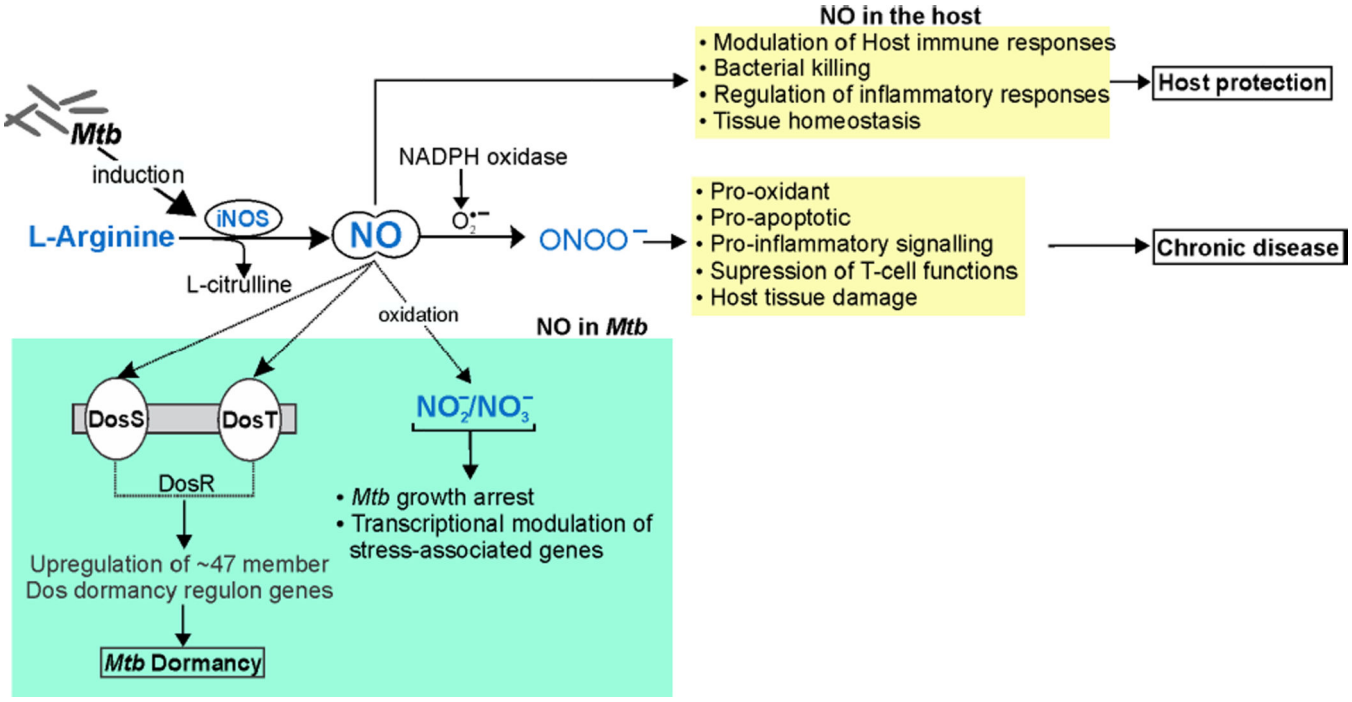

Figure 3. Schematic illustration of the role of NO in *Mtb* disease

Mtb infection upregulates the expression of iNOS, which catalyzes the conversion of L-arginine into L-citrulline and NO. NO-mediated *Mtb* killing and modulation of host immune and inflammatory responses are crucial for host protection against *Mtb* infection. On the other hand, NO reacts with $O_2^{\bullet-}$ to produce $ONOO^-$ via NADPH oxidase. $ONOO^-$ is pro-inflammatory, pro-oxidant and a potent suppressor of T-cell responses and its overproduction leads to host tissue damage and increased disease pathology. NO also plays important roles in *Mtb* physiology where it is sensed by the *Mtb* heme sensor kinase proteins DosS and DosT, which activate the response regulator DosR thereby inducing the *Mtb* dormancy regulon. Also, NO can be converted to NO_2^- or NO_3^-, which arrest *Mtb* growth and lead to transcriptional adaptation of *Mtb* stress-associated genes.

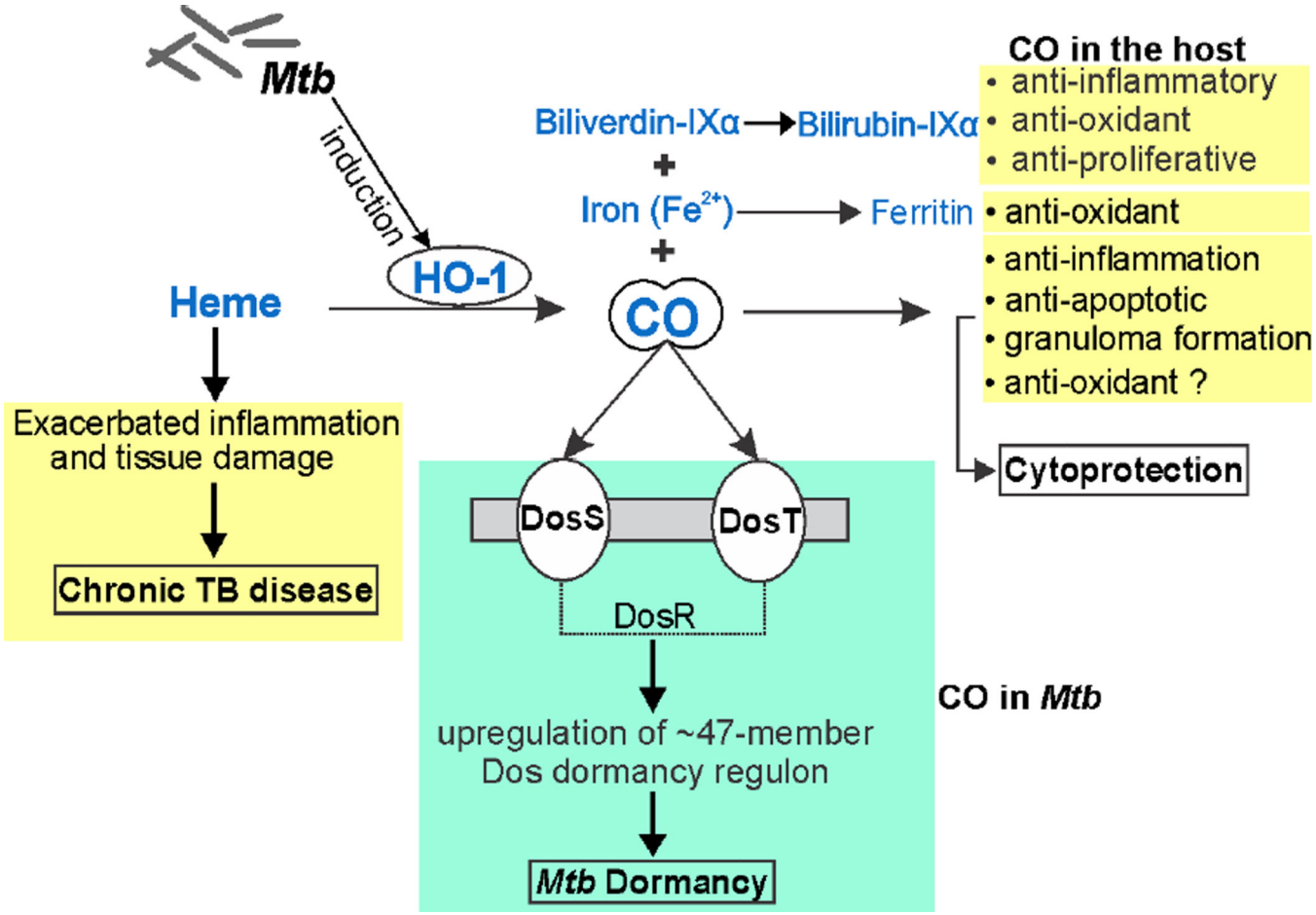

Figure 4. Schematic illustration of endogenous CO production and role in *Mtb* disease

CO is produced by HO-1 via heme catabolism. Other products of this enzymatic reaction include free iron (Fe^{2+}) and biliverdin, which is further converted into bilirubin. Iron further increases the expression of ferritin, an iron storage protein. CO, bilirubin and ferritin are potent antioxidant molecules. Further, CO and bilirubin also play important roles in regulating inflammation, immune cell proliferation and host cell apoptosis. *Mtb* infection induces HO-1 expression thereby increasing endogenous CO, which could protect against heme-mediated tissue damage via its anti-oxidative, anti-proliferative and anti-apoptotic properties. CO binds to *Mtb* heme sensor kinase proteins DosS and DosT, which like NO, induce the *Mtb* dormancy regulon.

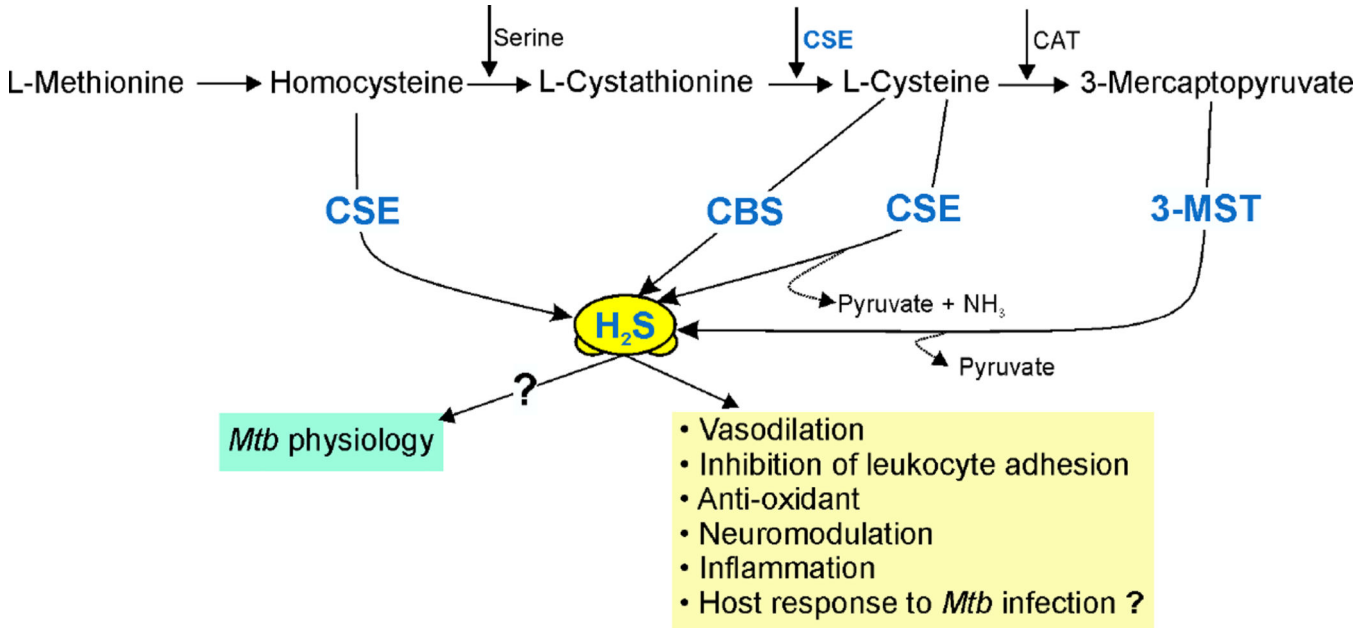

Figure 5. Schematic illustration of endogenous H_2S production and functions

H_2S is produced endogenously by cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). CBS and CSE use L-cysteine as the primary substrate with pyridoxal-5′-phosphate as a cofactor. H_2S formation through 3-MST is downstream of cysteine aminotransferase (CAT), which catalyzes the reaction of L-cysteine with α-ketoglutarate leading to the formation of 3-mercaptopyruvate, the substrate used by 3-MST to form H_2S and pyruvate. H_2S is important for numerous physiological responses including vasodilation, neuromodulation, and inflammation and can also act as an anti-oxidant.

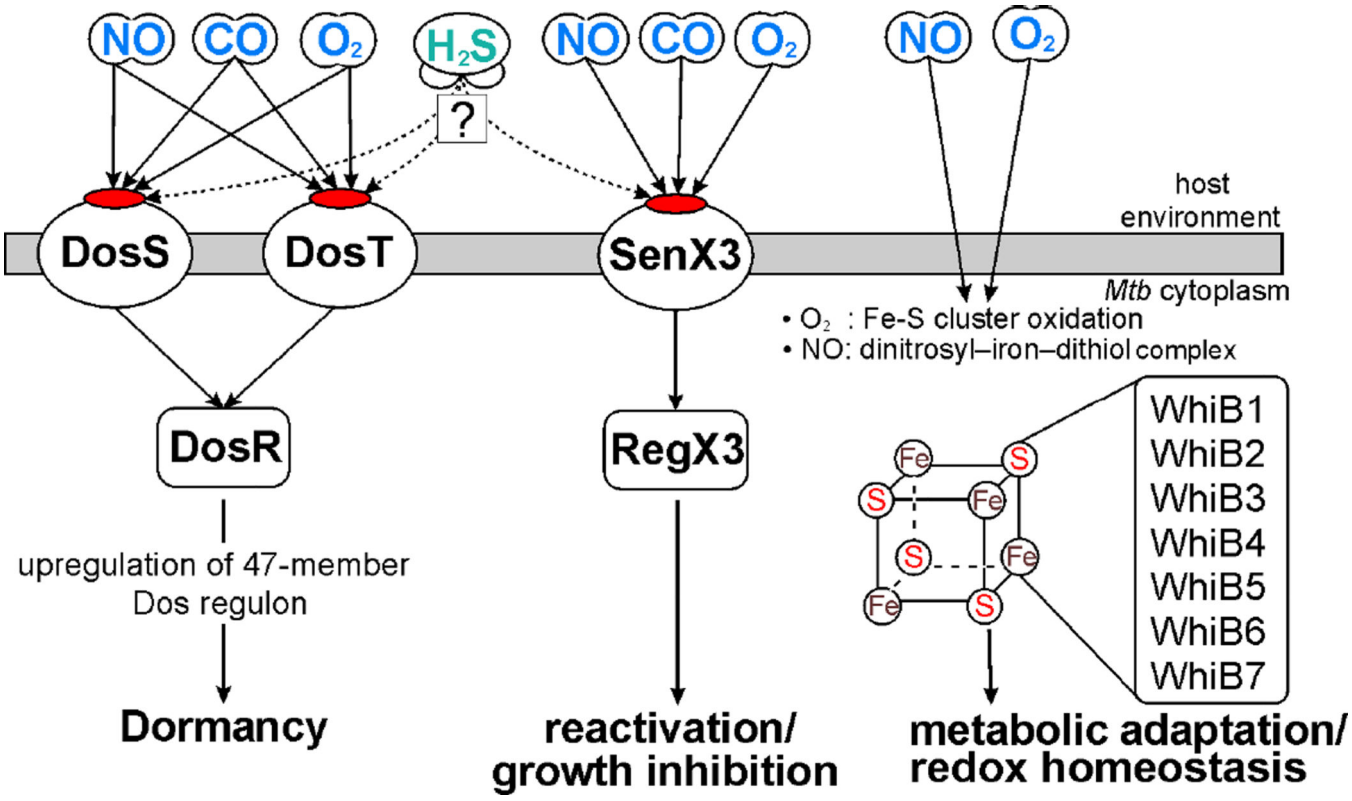

Figure 6. Schematic overview of gasotransmitters in *Mtb* physiology

Mtb heme sensor kinase proteins DosS and DosT sense host-generated NO, CO and O_2 and activate the response regulator DosR which induces the *Mtb* dormancy (Dos) regulon. SenX3 of the SenX3-RegX3 two-component system is a heme sensor kinase that also responds to host-generated O_2, NO and CO. Binding of O_2 to SenX3 stimulates *Mtb* growth during reactivation; however, binding of CO or NO inhibits growth. RegX3 is presumed to mediate these effects downstream of SenX3. Further, interaction of O_2 or NO with members of the WhiB family of iron-sulfur cluster proteins regulates the intracellular redox homeostasis of *Mtb* as well as metabolic adaptation under conditions of host-generated stress.