Chapter

Superoxide Dismutase: A Key Enzyme for the Survival of Intracellular Pathogens in Host

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Abstract

Superoxide dismutase (SOD) is a crucial enzyme required to maintain the redox potential of the cells. It plays a vital role in protecting normal cells from reactive oxygen species (ROS) produced during many intracellular pathogens infections. SOD removes excess superoxide radicals (O^{2-}) by converting them to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Several superoxide dismutase enzymes have been identified based on the metal ion as a cofactor. Human SOD differs from the intracellular pathogens in having Cu/Zn and Mn as metal cofactors. However, SOD of intracellular pathogens such as *Trypanosoma, Leishmania, Plasmodium*, and *Mycobacterium* have iron (Fe) as metal cofactors. Iron Superoxide Dismutase (FeSOD) is an essential enzyme in these pathogens that neutralizes the free radical of oxygen (O^-) and prevents the formation of Peroxynitrite anion ($ONOO^-$), helping the pathogens escape from redox-based cytotoxic killing. Moreover, most intracellular bacteria hold MnSOD or FeSOD in their cytoplasm such as *Salmonella* and *Staphylococcus*, whereas periplasm of some pathogenic bacteria and fungi are also cofactors with Cu/Zn and identified as CuZnSOD. This chapter will review the various types SOD present in intracellular pathogens and their role in the survival of these pathogens inside their host niche.

Keywords: Superoxide dismutase, Intracellular Pathogen, Reactive oxygen species, Antioxidant enzyme

1. Introduction

Reactive oxygen species are primarily the result of the by-product of the redox process and may also be produced to initiate intracellular signaling and antimicrobial activity. The general phenomenon is to maintain the ROS level in the cell by antioxidant enzymes and antioxidants molecules present in cells [1]. One of the prime sources of ROS in mammalian cells is the respiratory chain in mitochondria. It's well established that ROS generation is an essential modulator of inflammatory reactions in mammals. The enzyme NADPH oxidase induced the oxidative burst, leading to a dramatic increase in oxygen consumption and increasing the phagocytosis process. Activated macrophage induced the expression of IFN-γ and TNF-α cytokines,

improving NADPH oxidase activity resulting in ROS production, such as oxygen-free radicals ($O_2^{\bullet-}$) superoxide. The $O_2^{\bullet-}$ species are converted into hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) by spontaneously or enzymatic reaction [2, 3]. Activation of nitric oxide synthase (iNOS) or (NOS_2) protein in macrophage stimulates the increased secretion of nitric oxide (NO) and $^{\bullet}NO$-metabolite levels within the cell. ROS is the first superoxide radicals produced by mitochondria. ROS is a highly reactive oxygen species and does not diffuse quickly from cells since the leading site of ROS production is in the inner mitochondrial membrane. H_2O_2 is derived from mitochondrial ROS and detoxified by superoxide dismutase. ROS detoxification has been assigned in ROS-generating sites in the cell, such as mitochondria, glycosome, endoplasmic reticulum, and cytosol. Hydrogen peroxide (H_2O_2) is not considered a free radical by definition since it lacks free electrons. Still, NO is deemed to be free radicals, has also been involved in ROS-mediated damage. However, NO has a dual nature, like as beneficial as well as vicious [4–6].

Aerobic organisms exhibited two major antioxidant defense systems to minimize the ROS-mediated damage occurring due to oxygen-free radicals. The first one is enzymatic defense, and the second is low molecular weight antioxidants such as vitamins and phytochemicals. In general, cells control oxidative stress by three essential antioxidant enzymes which are present in it; (i) Superoxide dismutase is a class of oxidoreductase enzymes that contain metal ions in their active site (Fe or Mn and/or Cu/Zn) and is responsible for converting superoxide anion into H_2O_2. (ii) glutathione peroxidase is responsible for the reduction of H_2O_2 into hydroperoxides using glutathione as hydrogen donor, and (iii) catalase is responsible for the breakdown of H_2O_2 into O_2 and H_2O [7]. Since the activity glutathione peroxidase is required glutathione as hydrogen donor. Thus, the NADPH-dependent reduction of oxidized glutathione to maintain a steady state of glutathione is needed for GSH activity [1].

Superoxide dismutase catalyzes the dismutation of oxygen free radical to O_2 and H_2O_2 in the cell. SOD enzymes also participate in signaling pathways by controlling ROS action and protecting the cells from the toxic effects of superoxide radicals. Intracellular SODs mainly restrict superoxide action, which harms the cells by damaging the Fe-S cluster-containing enzymes. Extracellular SODs also guard the cells from superoxide released by the host or pathogens. For example, extracellular SODs of microbial pathogens are protected by ROS-mediated killing of host cells. The host cells antioxidant system includes enzymes such as SOD, catalases, and peroxidases [8, 9].

2. Superoxide dismutase

The evolutionary history of metalloenzyme superoxide dismutase (SOD) is aged and has been there before the differentiation of eubacteria from archaea bacteria. It is ubiquitous protein present in all living organisms and plays a vital role in the extreme pressure defense against superoxide radicals in the cell. The SOD catalyzes the conversion of the two molecules of virulent oxygen free radical (O^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) by using two equivalents of H^+ ions [10]. SOD is marked as a strong free radical scavenger that can eliminate the toxic effects of superoxide produced during the reduction of molecular oxygen. SODs enzyme family have been classified based on several factors, and one is on the metal ion. In general, SODs contain the metal cofactor at their catalytic core and are classified into three major groups: copper/zinc (Cu/Zn-SOD) [11, 12], manganese (Mn-SOD) [13], and iron (Fe-SOD) [14–16]. SOD containing MnSOD, FeSOD and CuZnSOD are encrypted

by the gene sodA, sodB, and sodC, respectively. Nickel (Ni)- and iron-zinc (Fe/Zn) containing isozymes have also been identified in several bacteria [17, 18]. FeSOD has mainly reported in prokaryotes except few protozoan parasites, whereas MnSOD and CuZnSOD are found in both prokaryotes and eukaryotes. All these isoforms were identified based on their diverse sensitivities to cyanide (CN) and H_2O_2. The Cu, Zn-SOD is extremely sensitive to CN and H_2O_2 [19]. Mn-SOD is insensitive to CN and H_2O_2 [20], while Fe-SOD is not sensitive to CN but sensitive to H_2O_2 [21]. In addition, Mn-SOD and Fe-SOD, both were inhibited by chloroform–ethanol, but Cu, Zn-SOD is insensitive [22].

Moreover, SODs of intracellular bacteria are further classified into three groups based on their localization; Mn- and Fe-cofactor SODs are found in the cytosol. In contrast, the third one of SOD cofactor by Cu-Zn and is attached with periplasm or anchored with the lipid of the outer envelope [23, 24]. Cu/Zn-SOD of bacteria dismutase superoxide produced by host cell during phagocytosis contributes to helping bacterial virulence [25, 26]. Additionally, few families of SODs also use a Ni ion as cofactor at their catalytic core to initiate its functions [27]. A study has shown that superoxide dismutase from *Streptococcus* is capable of making a cofactor substitution with Fe in place of Mn [28]. On the other hand, *Leishmania tropica*, *Trypanosoma brueci*, and *Crithidia fasciculate* have superoxide dismutase, which is insensitivity to cyanide but sensitive to azide and peroxide [29]. SODs of Trypanosomatids are having Fe as a metal cofactor at their catalytic core and are categorized as iron superoxide dismutase (Fe-SOD). Other protozoan parasites also have the same Fe-SOD, such as *Plasmodium falciparum* and *Entamoeba histolytica*, where enzyme-mediated free radical catabolism is fully Fe-SOD dependent [30]. Fe-SOD isoform was first discovered in *Escherichia coli* in 1973 by Yost and Fridovich. Subsequently, the same isoform was characterized in *T. cruzi* in 1977. Like Trypanoredoxin (TR), SODs of *T. cruzi* differ from the mammalian host. Trypanosomatids, other protozoan parasites (*P. falciparum and E. histolytica*), some plants, and Archaea possess only Fe-SOD. However, humans and other mammalian hosts contain Cu/Zn-SOD and Mn-SOD as core metal **Figure 1** [31].

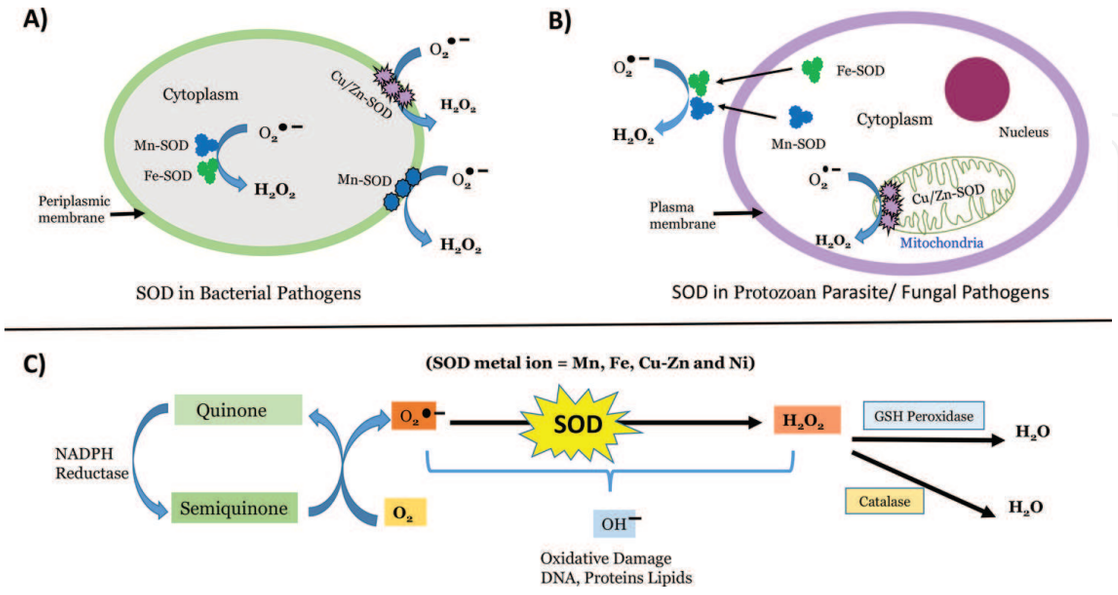

Figure 1.
Schematic representation of SOD localization in intracellular pathogens and SOD chemical reaction. A) Localization of SOD in bacterial pathogens, B) localization of SOD in protozoan and fungal pathogens and C) SOD reaction in mitochondria of protozoan and fungal pathogens.

This chapter will discuss role of superoxide dismutase in various intracellular pathogens that are belong to protozoan parasites genus *Trypanosoma, Leishmania, Plasmodium* and *Toxoplasma*, bacterial intracellular pathogens belongs to genus *Mycobacterium, Salmonella, Francisella* and *Staphylococcus* and fungal intracellular pathogens belongs to genus *Cryptococcus and Histoplasma* etc.

3. Role of SOD in intracellular parasites

There are several intracellular protozoan parasites which are causing severe illness in human's beings and if left untreated 100% mortality. These intracellular parasites belonging to the genus *Plasmodium*, *Leishmania,* and *Trypanosoma*, causing a spectrum of diseases like malaria, Leishmaniasis, African sleeping sickness, and Chagas disease in humans [1]. Antioxidant defense of pathogenic protozoan parasites is significantly distinct from each other as well as compared to their mammalian host. Trypanosomatids, as well as *Plasmodium* species have an Fe-containing SOD isoform, which is typically found in bacteria but absent in other eukaryotic cells [32, 33]. The main function of Fe-SOD is to neutralizing the $O^{[-]}$ that are formed during the generation of the superoxide radical [34]. Parasite persistence is determined by a balance between the ability of the immune response and resistance against free radicals produced by host cells. *Leishmania*-infected macrophages are able to produce inflammatory cytokines, ROS, and $^{\bullet}NO$ derivatives, which usually lead to the killing of the phagocytosed microorganism. However, *Leishmania* and *Trypanosoma spp*. are few protozoa that can survive and resist cytotoxic environments within the macrophage, and further, they can able to replicate in such a hostile condition **Table 1** [4, 5].

3.1 Trypanosomiasis

Chagas is a parasitic disease caused by intracellular parasites *Trypanosoma cruzi*. The prevalence of the disease is around 6–7 million worldwide, mainly in Latin America and listed in 17 neglected tropical diseases (NTD) classified by the WHO (WHO-2021). The present chemotherapy is relay on two available drugs 5-nitrofuran-nifurtimox (NFX) and 2-nitroimidazole benznidazole [65]. *T. cruzi* contains only Fe-dependent superoxide dismutase (Fe-SOD). Parasites have two dimeric Fe-SOD isoforms, one mitochondrial and one cytosolic isoform. However, Mateo et al. [35] investigated and characterized 4 Fe-SODs in *T. cruzi* epimastigotes, mainly cytosolic. The level of Fe-SOD increases during the differentiation of short stumpy forms of the parasite into dividing procyclic forms [66]. Therefore, Fe-SODs could be a promising drug target for the development of anti-chagasic drugs because of their exclusivity in *T. cruzi*. Furthermore, the crystal structures of the cytosolic Fe-SOD and the mitochondrial Fe-SOD from *T. cruzi* suggest that each enzyme has two polypeptide chains and two active sites composed of a Fe2+/Fe3+ ion, respectively. In Chagas disease, phagocytosis of parasites by macrophages is the first line of defense against the parasites by the host. Macrophage produces superoxide radical ($O2^{\bullet-}$), which diffuses into parasitophorous vacuoles, causing toxic environments to the parasites. However, *T. cruzi* is also equipped with an antioxidant network to counter the host-derived ROS activity. During infections, parasites are internalized into the phagolysosomal compartment and activate the NADPH oxidase 2 complex (Nox2) of the host macrophage [67]. Nox2 activity in macrophages results in intraphagosomal formation of oxygen free radicals ($O2^{\bullet-}$) and $O2^{\bullet-}$ derived ROS, which is required to

Infectious Group	Disease	Agent	Major metal ions	Sub-class of SOD	Role of SOD in Pathogenesis	Reference
Parasitic disease	Trypanosomiasis	*Trypanosoma cruzi, T. bruci*	Fe-SOD	SOD-B1 & B2, SOD-A & SOD-C	Increase the resistance of parasite and decrease ROS mediated phagocytic killing	[30, 35]
	Leishmaniasis	*Leishmania major, L. donovani, L. tropica, L. major, L. chagasi*	Fe-SOD	SOD-A, SOD-B1 & B2	Increase the virulence of the parasites and decrease ROS-mediated phagocytic killing	[36–39]
	Malaria	*Plasmodium falciparum, P. ovale, P. malariae, P. vivax*	Fe-SOD	SOD-1 & 2	Limit the toxicity of ROS produced during hemoglobin degradation	[40–43]
	Toxoplasmosis	*Toxoplasma gondii*	Fe-SOD	SOD-B1, SOD2 & SOD3	Increased the intracellular growth of parasites. Triggered the humoral and cellular immune responses	[44–46]
Bacterial disease	Tuberculosis	*Mycobacterium tuberculosis, M. leprae*	Fe-SOD, Cu/Zn-SOD	SOD-B SOD-C	Inhibits the iNOS activity, IFN-γ expression & control apoptosis and TLR2 expression and signaling	[25, 47, 48]
	Salmonellosis	*Salmonella typhimurium,*	Mn-SOD, Cu/Zn-SOD	SOD-A SOD-C	Neutralizing the ROS mediated activity and inducible nitric oxide synthase activities and increase the virulence	[49–52]
	Tularemia	*Francisella tularensis*	Fe-SOD, Cu/Zn-SOD	SOB-B, SOD-C	Limits the iron requirement to produce the highly lethal OH· free radicals. Increase the virulence of bacteria	[53–56]
	Boils and Toxic shock syndrome	*Staphylococcus aureus*	Mn-SOD Fe-SOD	SOD-A SOD-M	Increase the resistance to oxidative stress, and induced virulence and infection	[57–59]
Fungal Disease	Cryptococcosis	*Cryptococcus neoformans*	Zn-SOD, Mn-SOD	SOD1 SOD-2	Increase the virulence factor and menadione resistance	[60–63]
	Histoplasmosis	*Histplasma capsulatum*	Cu/Zn-SOD	SOD-1 SOD-3	Decrease the ROS-mediated oxidative killing.	[64]

Table 1.
Distribution of superoxide dismutase (SOD) & their sub-class in various intracellular pathogens and their role in pathogenesis of respective diseases.

neutralize parasite proliferation and disrupt its differentiation in the early stage of infection. Macrophages derived from Nox2-deficient (gp91phox−/−) mice produced marginal amounts of superoxide radical and are more susceptible to parasite infection than those macrophages derived from wild-type mice. Nox2-derived superoxide radical plays a crucial role in controlling *T. cruzi* infection in the early phase of a murine model of Chagas disease [68]. Inhibition or ablation of the Nox2 enzyme has shown to be detrimental for controlling the infection of a number of pathogens *in vitro* and *in vivo* [69, 70].

Trypanosoma brucei is an obligate intracellular protozoan parasite that causes sleeping sickness in humans in many countries of sub-Saharan Africa. Various sub-species of parasites cause the disease and responsible for more than 90% of all trypanosomal diseases in humans [71]. Overexpression of SOD-B1 in *T. brucei* has shown hypersensitivity to a trypanocidal agent such as benznidazole and gentian violet. A similar study in *L. chagasi* revealed that an increase in SOD-B1 protein leads to resistance toward paraquat and nitroprusside [72]. Deleting one copy of Sod-B1 gene in the *L. chagasi* increased the sensitivity to the drug and a significantly decreased the parasites survival within the host macrophage. *T. brucei* serves four SOD isoforms, of which three are iron-dependent, which is typically very much similar to prokaryotic SODs. Localization studies reveal that out of four SOD, two are predominantly found in the glycosome (*Tb*SOD-B1 and *Tb*SOD-B2), and the other two are found in mitochondria (*Tb*SOD-A and *Tb*SOD-C) [30]. Overexpression of cytosolic Fe-SOD-B of *T cruzi* showed more resistance to the phagocytic killing of macrophages and increased intracellular proliferation than wild-type (WT) parasites. Fe-SOD-B overexpressed mutant parasites showed higher infectivity than WT but lost in gp91-phox−/− macrophages, emphasizing the role of $O2^{\bullet-}$ in parasite killing [67]. *Tc*FeSOD-A gene amplification increases the *Tc*FeSOD protein expression and enzyme activity in a *T. Cruzi* induced resistance to benznidazole and gentian violet treatments [73]. The reduced expression of *Tb*SOD-B leads to rapid accumulation of superoxide anion within the trypanosome responsible for detoxifying highly toxic radical in the parasite [74].

3.2 Leishmaniasis

Leishmaniasis is an intracellular protozoan disease caused by *Leishmania* parasites. Leishmaniasis is usually prevalent in tropical and subtropical regions of the world [36, 75]. *Leishmania* parasite infects host macrophages, survives in parasitophorous vacuoles of the macrophage, and escapes from the oxidative killing of the parasite by neutralizing the ROS activity. *Leishmania* Fe-SOD can be classified into two types based on their localization: FeSOD-A isoform is localized in mitochondria, and is related to cellular respiration; FeSOD-B1 and FeSOD-B2 are localized in glycosomes and reduce the oxidative stress generated from cellular reactions [37]. *L. major* contains Sod-B1, Sod-B2, and Sod-C genes on chromosome 32 and sod-A gene on chromosome 8. Sod-B1 and Sod-B2 genes are organized in tandem in both *L. chagasi* and *L. donovani*. Metacyclic promastigote of *L. amazonensis*, when lacking one allele of the Sod-A gene, failed to replicate in macrophages and severely attenuated their ability to established the cutaneous lesions in mice. In addition, the reduction of SOD-A expression in parasites resulting in increased susceptibility to oxidative damage. The failure of SODA/sod-A functions in promastigotes compromised their differentiation

into axenic amastigotes. Hence, SOD-A promotes *Leishmania* virulence by protecting the parasites against oxidative stress and initiating ROS-mediated signaling mechanisms, which are required to determine infective forms [37]. *L. chagasi* SOD-B1 null mutant parasites are not viable inside host macrophages. Furthermore, parasites lacking one SOD-B1 allele have markedly reduced their viability [38]. Moreover, WT and SOD-B1/Δsodb1 *L. major* promastigotes have equal capacity to establish infection in murine bone marrow macrophages. However, in contrast to WT parasites, *L. major* SOD-B1/Δsodb1 deficient parasites are declined in number over time in macrophages. The results suggesting its normal level of SOD-B1 is required for *L. major* endurance in macrophages and virulence in mice [76]. The Fe-SOD transcript level and enzyme activity are higher in the amastigote than in the promastigote stage of the parasite when treated with nitroprusside and parquet in *L. chagasi* [72]. In *Leishmania*, FeSOD-A appears to be the first line of defense against ROS and is crucial for parasite survival inside macrophages. Antimony (SbIII) resistant *L. (Viannia) brazilensis* (LbSbR) and *L.* (Leishmania) *infantum* (LiSbR) lines express higher FeSOD-A specific enzyme activity compared to wild type control and showed more resistance toward Antimony (SbIII) [77, 78]. Moreover, miltefosine resistant *L. donovani* are able induce the overexpression of *Ld*FeSODA to protects from drug-induced cytotoxicity, reduces superoxide generation, and involves in suppression of oxidative stress-induced programmed cell death by reducing the phosphatidylserine exposure, DNA damage [79, 80]. Increased exposure of *L. donovani* to miltefosine makes resistance due to the release of *Ld*FeSOD-A into the cytosol from mitochondria. This release of *Ld*FeSOD-A into the cytosol or the inhibition of *Ld*FeSOD-A import into the mitochondria makes the mitochondria even more susceptible to oxidative stress due to the accumulation of ROS. Mitochondria of the parasite are more vulnerable to ROS, leading to programmed cell death, emphasizing its role in keeping healthy mitochondria [39].

3.3 Malaria

Malaria is caused by an intracellular protozoan parasite belongs to the genus Plasmodium. Malaria is endemic in most of tropical countries and subtropical regions of Asia, Africa, South, and Central America. Plasmodium can differentiate and replicate inside hepatocytes, and then released as merozoites into the bloodstream, which subsequently invades red blood cells (RBCs) [81]. Plasmodium parasite uses SOD to reduce the toxicity of ROS throughout the intra-erythrocytic stage of parasite survival. The SOD activity in *Plasmodium falciparum* and rodent malaria species is characterized as iron-dependent and the first level of the antioxidant defense system of the parasite [40, 81, 82]. *P. falciparum* consists two distinct genes coding for different SOD such as *Pf*FeSOD-1 and *Pf*FeSOD-2 [40]. *Pf*FeSOD-1 is a cytosolic protein and expressed during the intra-erythrocytic cycle of the parasite [41, 83]. FeSOD-1 is also reported in *P. ovale*, *P. malariae*, and *P. vivax* and very close apicomplexan parasites such as *Toxoplasma gondii* [42]. Since FeSOD-1 is a cytosolic protein, it is unlikely to act on a superoxide anion in the parasite food vacuole during hemoglobin digestion. Thus, it is plausible that parasites might be taking a large amount of Cu/Zn-SOD from the host erythrocyte to detoxify the superoxide anions in their organelles [84]. Plasmodium parasite utilizes SODs enzymes to limit the toxicity of ROS produced during hemoglobin degradation in the erythrocytic cycle. These enzymes play a crucial role in parasite persistence and their intracellular survival during the

intra-erythrocytic stage of the life cycle. FeSOD1 of *Plasmodium vinckei* (PvSOD1) also plays a central role in the oxidative defense of these parasites. However, PvSOD1 is inhibited by H_2O_2 and peroxynitrite, but not by cyanide and azide [85]. The FeSOD-2 of *P. falciparum* is a mitochondrial SOD with an elongated N-terminal protein extension, reminiscent of a bipartite apicoplast-localized protein [43, 86]. An inhibition study of recombinant *P. falciparum* FeSOD suggested that SOD is a highly selective drug target to designed antimalarial drugs. The study further identified many antimalarial drugs which have shown antimalarial activities against *P. falciparum and even* a strain moderately resistant to chloroquine [87].

3.4 Toxoplasmosis

Toxoplasma gondii is an obligate intracellular protozoan pathogen that infects nearly all warm-blooded animals. Toxoplasmosis is one of the most prevalent parasitic diseases, an estimated one-third of the global population are at risk. Still, it is considered a neglected parasitic disease [88]. *T. gondii* causes life-threatening illnesses in developing fetuses and in persons with immunocompromised [89]. In chronic infection, *T. gondii* spreads in various organs such as the heart and brain through the circulatory system [90]. *T. gondii* RH tachyzoites treated with resveratrol and pyrimethamine significantly increased SOD activity to restrain ROS action for their survival [44]. Interestingly, human macrophages failed to produced ROS during *T. gondii*-infection [91], possibly due to an immune evasion mechanism of parasites. *T. gondii* targets the host NADPH oxidase enzyme by reducing the expression of Nox4 transcript and protein, resulting in diminished the release of intracellular ROS. In infected cells, Nox4 gene expression was associated with activation of PI3K/AKT signaling [92]. However, superoxide dismutase and catalase enzymes might be playing a role in intracellular survival but, it does not have a basis for differences in virulence to mice [93]. *In T. gondii,* SODs are found in nearly all developmental stages of parasites, suggesting their importance in detoxifying superoxide radicals to protect the parasite. *T. gondii* contains three types of SOD; SOD-B1 (Fe-SOD), different from the Mn-binding SOD of humans. SOD-B1 is a cytoplasmic and essential enzyme, and SOD-B1 gene knock-outs lead to be lethal for parasites [94, 95]. SOD2 and SOD3 are found in the mitochondria of parasites and have conserved residues to bind iron. However, they are very similar in the primary sequence to SODs from *P. falciparum* [45] *T. gondii* superoxide dismutase (*Tg*SOD) also affects the intracellular multiplication of both bradyzoite and tachyzoite forms of parasites. A recombinant DNA vaccine containing the antigen gene of *T. gondii* were elicited high levels of antibodies, a Th1 type of immune response with significant production of IFN-γ, and low levels of IL-4 or IL-10 in BALB/c mice [96]. Moreover, a DNA vaccine containing the TgSOD gene triggered potent humoral and cellular immune responses, and it stimulates biased protective immunity against acute *T. gondii* infection in BALB/c mice [46]. SOD-DNA vaccines of L. amazonensis immunized mice were partially protected from parasites once challenged. Mice showed a mixed immune response, including the production of IFN-γ and IL-4 from CD4+ and CD8+ T lymphocytes [69]. In addition, the SOD vaccine of *Brugia malayi* was also shown to trigger a typical Th1 response against infective larvae and microfilariae in jirds with filarial infection [97]. The above finding reveals that SOD-dependent vaccines have potential vaccine efficacy, either by protein or DNA-based vaccines, to control intracellular pathogen by activating the protective Th1 type of immune responses in animals.

4. Role of SOD in intracellular bacteria

There are several intracellular bacteria which are causing severe illness in human's beings and if left untreated 100% mortality. Most pathogenic bacteria contain MnSOD or FeSOD in their cytoplasm, while CuZnSOD has been found on the periplasm of pathogenic bacteria and played an essential role during phagocytosis [11, 23]. In addition to their ability to detoxify free radicals during aerobic growth, bacterial SODs are also critical in determining the virulence factors. In several intracellular bacterial infections, SOD-C acts as a critical virulence factor, and its localization to the periplasmic membrane protects bacteria from ROS derived from host cells [49, 98–100]. Moreover, many virulent bacteria maintain two copies of the sodC gene [101]. The evolutionary maintenance of an extra sodC gene copy indicates that SOD is essential for pathogenic bacteria for their survival inside the host niche [101]. These pathogens belong to the categories of genus *Mycobacterium, Salmonella, Staphylocccus* and *Francisella*, causing spectrum of disease like tuberculosis, leprosy, typhoid, boils, furuncles, cellulitis and tularemia etc. **Table 1**.

4.1 Tuberculosis and leprosy

Mycobacterium is an intracellular bacterium, which is causing two distinct disease manifestations in humans, such as Tuberculosis and Leprosy. Tuberculosis (TB) is caused by *M. tuberculosis*, a leading infectious agent that claims millions of deaths worldwide/year [102]. *M. tuberculosis* is encountered several exogenous and endogenous redox pressures throughout its pathogenic life cycle. Therefore, they use various in-house enzymes to detoxify and neutralize the redox potential produced by host cells. Catalase–Peroxidase, Superoxide dismutase, and Alkyl Hydroperoxidase are the enzymes involved in the clearance of oxidative stress [47].

M. Tuberculosis is a highly pathogenic bacterium contains Fe-SOD and expresses 93-fold more superoxide dismutase. In contrast, non-pathogenic mycobacterium *M. Smegmatis* has Mn-SOD, and *M. Tuberculosis* export more enzyme than *M. smegmatis* [48]. Superoxide dismutase (SOD) of *M. tuberculosis* is a 207-residue enzyme with molecular mass of 23 kDa [103]. Treatment with diethyldithiocarbamate, a potent inhibitor of SOD, increased *M. lepraemurium* survival in murine splenic macrophages [104], suggesting that SOD protein is required for the long-term survival of mycobacterium in vivo [104] *M. tuberculosis* has two distinct SOD proteins, SOD-A and SOD-C. SOD-A is one of the main extracellular proteins contains Mn, Fe-SOD. SOD-C is much lower protein contains Cu, Zn SOD, and present in the outer membrane of the bacteria. SOD-C was upregulated during phagocytosis by macrophage, suggesting its importance in protecting the *M. tuberculosis* membrane against damage from superoxide radicals [25]. SOD of *M. tuberculosis* scavenge oxygen free radicals and inhibits the release of NO by inhibiting iNOS activity. It impairs acquired by down-regulating the IFN-γ expression as well as control the caspase-dependent apoptosis. SOD also inhibits innate immunity by down-regulating TLR2 expression as well as control the TLR2 dependent signaling in the cells [104].

Mycobacterium leprae is the causative agent of leprosy or Hansen's disease. *M. leprae* is the single known bacterial pathogen that infects superficial peripheral nerves. It is an intracellular pathogen that infects both myelinated and nonmyelinated Schwann cells of the nerve and proliferates within the monocyte/macrophage series cells. Peripheral nerves are not protected from the immune response of host due to the blood–brain barrier [105]. Hence, the advantage of *M. leprae* is to escape from the

phagocytosis actions of the macrophage may be a critical factor in its pathogenicity [106]. The SOD activity of *M. leprae* is lower than the other mycobacteria species such as *M. lepraemurium, M. phlei* [107]. Therefore, the ability to clear the *M. leprae* infection via SOD pathway appeared to be a distinct mannerism and is not dependent on macrophage activation and differentiation.

4.2 Salmonellosis

Salmonella typhimurium is a facultative intracellular bacterium that resides within modified phagosomes in macrophage promotes replication and escape from killing by ROS [108]. *S. typhimurium* infects a wide range of hosts, including animals, humans, and poultry. *S. typhimurium* causes acute gastroenteritis in humans and typhoid-like disease in mice. If left untreated, 100% fatal [50]. *Salmonella* infects the epithelial wall of the intestine and escapes from the innate immunity and ROS activity of the host. The SOD of *S. typhimurium* protects the bacterium from excessive ROS activity produced outside or inside of the host cell [109, 110]. Thus, SOD was considered a critical factor for bacterial survival by neutralizing the ROS activity [111]. The sod-A gene inactivation in *Salmonella* species is connected with limited protection from ROS and decreased virulence during mice infection [26, 109]. sod-A-deficient bacterium displayed a slightly lower growth rate compared to the wild-type strain. The loss of the sod-A gene in mutant bacteria harms the ability to infect the host cell. Consequently, the sod-A mutant bacterium is highly susceptible to the bactericidal action of host cells and has also shown attenuated virulence properties. More specifically, SOD-A plays a vital role in biofilm formation, increased resistance against oxidative stress, and overcome from bactericidal complement system of serum [51]. *Salmonella* combats phagocytic free radicals by producing the periplasmic superoxide dismutase. Periplasmic Cu, Zn-cofactor superoxide dismutase (SOD-C) protects *S. typhimurium* from extracellular phagocyte-derived oxidative damage by host cells. *Salmonella* deficient sod-C gene has shown abated survival inside the macrophage, increased ROS susceptibility, and attenuated virulence factor during in-vivo infection. Conclusively, SOD protects periplasmic or inner membrane targets by controlling the phagocytosis-dependent oxidative burst or inducible nitric oxide synthase activities during in vivo infection [49]. The evolutionary acquisition of the sod-C gene in *Salmonella* species extends an increased virulence trait of bacterium [52].

However, cytosolic Mn-SOD enzyme is essential for detoxifying intracellular superoxide radicals but not involved virulence [112]. SOD of *Streptococcus suis* resistant to anti-oxidative stress and ROS-generating herbicides, which is known to cause a severe damage to DNA, RNA, and proteins molecules that might contribute to its virulence in mice [53].

4.3 Tularemia

Francisella. tularensis is an intracellular pathogen that causes a disease called Tularemia. The disease is considered a potential biological threat for humans due to its extreme infectivity and substantial capacity to cause severe illness and death. The hallmark of the bacterium is their capability to survive and replicate within macrophages [113] and other cell types [114, 115]. The bacterium's survival depends on its ability to combat the microbicidal activity of macrophages such as ROS and reactive nitrogen species. *F. tularensis* require oxygen for their growth and possess ROS-scavenging enzymes such as super oxide dismutases, peroxidases, and catalases [116, 117].

Like other bacterial pathogens, *F. tularensis* contains two types of SOD gene: FeSOD (sod-B) and CuZnSOD (sod-C). SOD-B plays a dual role in protecting *F. tularensis* from the oxidative stress of the host. SOD-B binds to the iron with high affinity and limits the availability of iron requirement to produce the highly lethal OH·. Secondly, detoxification of superoxide prevents cellular damage of DNA, proteins, and lipids associated with O^{2-} toxicity [53, 54]. SOD-B dismutation decreasing the reaction of O2 with NO to form peroxynitrite (ONOO) and protect bacteria from ONOO- toxicity [55]. ONOO- has been shown to have a significant role in the IFN-γ -induced killing of *F. tularensis* (live vaccine strain) LVS by murine macrophages [99, 118]. However, the genome sequence of *F. tulrensis* LVS has possessed a single functional copy of the sod-B gene [117]. Hence, sod-B gene alteration leads to reduced SOD-B enzyme expression might be associated with high sensitivity to oxidative stress suggesting that sod-B is essential for bacterial survival under oxidative stress conditions. Therefore, increased survival of mice infected with sod-B mutant *F. tularensis* suggesting that SOD-B plays a role in virulence [56].

A recent study suggests SOD-C (CuZnSOD) of *F. tularensis* also plays a vital role in virulence factors. SOD-C is localized in the periplasm to protect from superoxide radicals (O^{2-}) derived from host cells. *F. tularensis* depleted sod-C (ΔsodC) mutant and *F. tularensis* ΔsodC mutant with attenuated sod-B gene expression (sodB ΔsodC) exhibited attenuated intracellular survival in IFN-γ-activated macrophages compared to the wild-type *F. tularensis* LVS. Transcomplementation of the sod-C gene in ΔsodC mutant bacteria or checking the IFN-γ-dependent production of O^{2-} or NO enhanced the survival of the sod mutant's bacteria in macrophage. The virulence capacity of the sodB ΔsodC mutant bacteria was significantly more attenuated as compared to ΔsodC mutant. Furthermore, lack of IFN-γ, iNOS, or PHOX restored the virulence of ΔsodC mutant strains, suggesting that the CuZnSOD of the bacterium is playing a critical role in restricting the bactericidal activities of ROS and RNS. The ΔsodC and sodB ΔsodC mutants were also significantly attenuated for virulence in intranasally challenged C57BL/6 mice compared to the wild-type *F. tularensis* LVS, indicating that SOD-C is required for resisting host-generated ROS and contribute to survival and virulence of *F. tularensis* in mice [119].

4.4 Staphylococcus (boils and toxic shock syndrome)

Staphylococcus aureus is a gram-positive bacterium, which causes a broad spectrum of diseases in humans. It is a facultative intracellular bacterium that invades and replicates within many types of phagocytic and non-professional phagocytes cells, such as endothelial cells, mammary cells, fibroblasts, and osteoclasts [120]. Bacterium commonly symptomatically colonizes in one-third of the population of the globe and is a leading cause of antibiotic-resistant [121]. Methicillin-resistant *S. aureus* (MRSA) strains are one of the utmost dangerous species and have shown resistance to all β-lactam antibiotics as well as other antimicrobials [122]. *S. aureus* is capable of subverting xenophagy and escaping from the cytosol of the host cell during intracellular infection [118, 122, 123]. During intracellular survival, *S. aureus* is capable to protects itself from the oxidative burst by numerous mechanisms, including enzymes such as SODs that detoxify the action of ROS activity [124, 125]. *S. aureus* serves two distinct SODs, SOD-A and SOD-M, both of which are cytoplasmic and reported as Mn-dependent [57, 126]. All Staphylococci species are contained SOD-A protein, while *S. aureus* also has a unique protein SOD-M [58]. The loss of either SOD-A or SOD-M in a skin model of infection or loss of both SODs in a systemic mouse model

of infection diminishes the ability of *S. aureus* to cause disease, highlighting the importance of SOD in the virulence [127, 128].

The lack of both SODs in *S. aureus* shown bacterium is more sensitive to host cells during manganese starvation, suggesting the importance of SOD in overcoming nutritional immunity. Mn starvation in host-mediated protein calprotectin reduces staphylococcal SOD activity during in vitro and in-vivo infection. Hence, Mn deficiency renders *S. aureus* more sensitive to oxidative stress and neutrophil-mediated killing [127, 129, 130]. SOD-A protein is essential for countering oxidative stress and disease progression when manganese is abundant. At the same time, SOD-M is important under manganese-deplete conditions. However, SOD-A is strictly manganese-dependent, whereas SOD-M contains either of two or more different metal atoms, having similar enzymatic activity when filled with manganese or iron. During host-dependent Mn starvation, *S. aureus* enables the ability of SOD-M to utilize Fe to retain its SOD activity. Subsequently, *S. aureus* enhances the ability to overcome nutritional immunity, resistance to oxidative stress, and ultimately induced virulence and infection [59].

5. Role of SOD in other fungal infection

Superoxide of pathogenic fungus are cofactors with Cu/Zn or Mn metals. The enzymes are localized in the cytosol as well as in mitochondria and involved in cell differentiation and multi-stress conditions. Mitochondrial Mn-SODs prevent the damages of oxidative stress, osmotic and thermal stresses in yeast cells. SODs protein has been shown to contribute to the virulence of many intracellular pathogenic fungi, such as *C. neoformans* [60], and *H. capsulatum*, both are capable to some degree of neutralizing the lethal levels of ROS produced by the host cells [64]. *C. neoformans* have Zn-SOD and Mn-SOD, while *H. capsulatum* has Cu/Zn-SOD. However, some fungal pathogens and fungal-like oomycetes have a unique SOD, such as Cu-SODs (SOD5). SOD5 are closely associated with the ubiquitous class of Cu/Zn-SODs but lack a Zn cofactor [34] and are believed to act on substrate level [131–133]. Unlike Cu/ZnSODs, which is found in both intra- and extracellularly, Cu-SODs are found exclusively in extracellular, and they appear primarily appended to the GPI anchors protein of cell surface [134, 135]. Cu-SODs have been proved to protect pathogens from the oxidative burst of the host regulated by immune cells [9] **Table 1**.

5.1 Cryptococcosis

Cryptococcus neoformans (*Cn*) is a facultative intracellular fungal pathogen and can propagate inside the host macrophages during many stages of experimental and human infections [136, 137]. *Cryptococcus* is a soil fungus that causes life-threatening meningitis in immunocompromised patients [138, 139]. *Cryptococcus* is an encapsulated pathogenic yeast composed primarily of glucuronoxylomannan (GXM). This polysaccharide helps the fungus play a defensive and offensive role during pathogenesis. It protects the fungus against phagocytosis and promoting intracellular pathogenesis through the cytotoxic release of polysaccharides into macrophage vacuoles [136]. *Cryptococcus* rarely causes clinically visible infections in healthy hosts, but it can be present in latency and persistence inside macrophages

[61, 62]. *C. neoformans var. gattii* predominantly infects individuals having a normal immune response, whereas var. grubii and neoformans are common in immunocompromised individuals. *C. neoformans var. gattii* hinders macrophage phagocytic response, whereas the other two varieties are readily killed by ROS released by phagocytic cells [140, 141].

C. neoformans is resistance to ROS mediated oxidative killing of macrophage by inducing the SOD activity and might be playing an important role in virulence of this fungus. Exogenous supplementation of SOD significantly controlled the bacterial growth by inducing human neutrophil function, suggesting that SOD plays a protective role during *C. neoformans* infection [63]. *Cryptococcus neoformans* var. *gattii* contains two types of SODs such as copper, zinc-depend SOD (SOD1) and Mn-dependent (SOD2) isoenzymes [142]. Both SOD1 and SOD2 are intracellular SODs, and deletion of their encoding genes reduces the fungal virulence in vivo model of infection. Furthermore, the mutant fungus also increases sensitivity to pharmacologically-induced intracellular oxidative stress [143]. The sod1 mutant *C. neoformans* was shown three characteristic features 1) highly sensitivity toward oxidative killing by human polymorphonuclear (PMN) cells and by the redox cycling agent menadione. 2) The sod1 mutant was markedly attenuated in virulence when raising the infection in mice, and it also showed significantly susceptibility to in vitro killing by human neutrophils. 3) SOD1 deletion also appeared to be defects in the expression of a number of virulence factors such as laccase, urease, and phospholipase. Complementation of the sod1 gene mutant *C. neoformans* with SOD1 protein regained the virulence factor and menadione resistance. Hence, the antioxidant function of SOD1 is critical for the pathogenesis of the fungus during intracellular survival [60, 141, 144].

5.2 Histoplasmosis

Histoplasma capsulatum is an intracellular fungal pathogen structurally similar to yeast cells. *H. capsulatum* successfully infect host cells like neutrophils and macrophages. *H. capsulatum* is prevalent in the Midwestern United States and Latin America. Macrophages efficiently phagocytize the *Histoplasma* cells, but they failed to kill the fungus despite having ample ROS production. *Histoplasma* cells counter the ROS-mediated oxidative stress of the host by three proteins that are possibly involved in defending *Histoplasma* from ROS. sod1 and sod3 gene deficient *Histoplasma* strains shown the spatial specificity of the SOD1 and SOD3 superoxide dismutases for internal and external (i.e., host-derived) superoxide, respectively. SOD-3 is the primary source of extracellular SODs, and its expression is significantly enriched in the pathogenic phase of fungus cells. *Histoplasma* SOD-3 offers higher resistance of fungus against the phagocytic killing of host cells leading to increased capacity to cause disease in immunocompetent hosts. In *in vivo* studies, sod-3 gene deficient *Histoplasma* strains were shown the attenuation in virulence in mice. Furthermore, restoration of Δsod3 mutant *Histoplasma* virulence in mice unable to produce superoxide radicals conclusively proves that SOD3 functions in the detoxification of superoxide generated by the host. SOD-3 also prevents the superoxide-dependent killing of *Histoplasma* yeast cells. The host to control the infection of *Histoplasma* requires ROS production. Hence, SOD-3 is a central virulence factor of *Histoplasma* and help to fungus survives under oxidative stress produced by host phagocytic cells during infection [64].

6. Conclusion

Superoxide's are the critical molecules produced by host cells to counter intracellular pathogens during infection. ROS is mainly produced within mitochondria of cells as byproducts of normal cell respiration. Defects in oxidative phosphorylation in cells could lead to an increase or decrease in ROS production by host cells. ROS-mediated destruction can directly affect the components of the electron transport system of host cells. Therefore, to reduce the ROS activity, host cells are evolved with three types of SODs such as NiSOD, Fe or MnSOD, and CuZnSOD to control the ROS activity produced by itself. More importantly, the immune cells of the host used ROS as defense molecules against various kinds of human pathogens during their infection.

Intracellular pathogens are also furnished with all types of SODs such as NiSOD, Fe or MnSOD, and CuZnSOD. Pathogens are using these SODs in neutralizing the free radicals produced by host cells during infection. SODs of intracellular pathogens can modulate the interaction with phagocytic cells at the onset of phagocytosis by altering the local concentrations of superoxide anion in parasitophorous vacuoles of host cells. SODs of these pathogens are also required to neutralized O2- generated by IFN-γ-activated macrophages, but not necessary for survival in quiescent macrophages. However, the role of SOD in combating other infection does not solely depend on the phagocytic ability of macrophages. In conclusion, SODs of intracellular pathogens are the key determinants of their survival inside the host niche. Furthermore, it also plays a vital role in the severity of disease and virulence of these pathogens by protecting them from extracellular host-derived ROS activity.

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