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Salmonella and Reactive Oxygen Species: A Love-Hate Relationship

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Abstract

Salmonella enterica represents an enterobacterial species including numerous serovars that cause infections at, or initiated at, the intestinal epithelium. Many serovars also act as facultative intracellular pathogens with a tropism for phagocytic cells. These bacteria not only survive in phagocytes but also undergo de facto replication therein. Phagocytes, through the activities of phagocyte NADPH-dependent oxidase and inducible nitric oxide synthase, are very proficient in converting molecular oxygen to reactive oxygen (ROS) and nitrogen species (RNS). These compounds represent highly efficient effectors of the innate immune defense. Salmonella is by no means resistant to these effectors, which may stand in contrast to the host niches chosen. To cope with this paradox, these bacteria rely on an array of detoxification and repair systems. Combination these systems allows for a high enough tolerance to ROS and RNS to enable establishment of infection. In addition, salmonella possesses protein factors that have the potential to dampen the infection-associated inflammation, which evidently results in a reduced exposure to ROS and RNS. This review attempts to summarize the activities and strategies by which salmonella tries to cope with ROS and RNS and how the bacterium can make use of these innate defense factors.

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Reactive Oxygen Species

Reactive oxygen species (ROS) are commonly present in various habitats occupied by living organisms. ROS are formed in the path of abiotic processes but also by living cells themselves, for example through photosynthesis and the activity of the respiratory electron transport chain in mitochondria and bacterial cytoplasmic membranes [1, 2]. As such, ROS cause damage to most, if not all, biomolecules [3, 4], including oxidation of amino acids, vitamins, lipids, nucleotides, and DNA, with damage to the later promoting mutations [5]. Indeed, deliberate production of ROS appears as a very ancient host strategy for coping with pathogens [6–11] and for acquisition of nutrients [12]. Upon contact with microbes, excessive production of ROS might also become detrimental to the host itself. For example, ROS contribute to endotoxemic shock [13, 14], whereas Jurkat T cells undergo necroptosis upon contact with pathogenic amoebae as a result of a

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strong ROS response [15]. Likewise, the nematode *Caenorhabditis elegans* is killed during infection with *S.* Typhimurium through a massive ROS response filling the whole nematode, almost resembling a primordial septic shock [16].

Even pathogens themselves might produce ROS to their own disadvantage. Several classes of bacteriocidal antibiotics, including cell wall synthesis inhibitors, have been suggested to induce ROS production in bacteria via triggering of the tricarboxylic acid cycle, and ultimately hyperactivation of the electron transport chain with concomitant ROS production [17]. Concomitantly it has been proposed that wall synthesis inhibitors, for example, in part mediate their antibacterial effect through endogenous ROS production. Intestinal bacteria will inevitably be exposed to bile. Interestingly bile induces production of ROS and a genetic ROS response signature in salmonella [18]. Host antibacterial peptidoglycan recognition proteins, an additional group of effectors in our innate immunity barrier, also induce ROS stress in target bacteria, which likely contribute to the antibacterial activity of peptidoglycan recognition proteins [19].

From the host side, be it a mammal or a plant, the NADPH-dependent oxidases (in mammals NADPH-dependent phagocyte oxidase [Phox]) act as a major source of superoxide [6]. In this molecular oxygen (O_2) is converted into superoxide anions (O_2^-) which may subsequently decompose into hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO\bullet$), and eventually water [3]. Also, phagocyte inducible nitric oxide synthase (iNOS) produce large amounts of the reactive nitrogen species (RNS) nitric oxide (NO) from L-arginine, NADPH, and molecular oxygen. NO may eventually be converted into peroxynitrite ($ONOO^-$) in the presence of ROS [20]. Apart from causing damage to biomolecules, NO also acts as a biological transmitter causing, among other things, vasodilation, a condition characteristic of septic shock. Thus, production of ROS and RNS may be useful arms in innate antimicrobial defense, but at the same time it is weaponry to be regulated and used with care. Likewise, successful pathogens somehow have to cope with ROS and RNS in order to prevail in a host.

Salmonella enterica and Salmonellosis

Many serovars of *Salmonella enterica* act as facultative intracellular pathogens that cause intestinal and invasive diseases in humans and animals. The infection is acquired via the oral route, whereafter the bacteria invade the intestinal epithelium. In human typhoid fever, caused by the human-specific serovar Typhi (*S.* Typhi), the bacteria proceed deeper to infect the liver, spleen, gall bladder, and bone marrow [21]. This form of salmonellosis is also called the typhoidal variant of the disease. Relapses and establishment of persistent carriage, in quite a proportion of the convalescents, are also hallmarks of typhoid fever [22]. Despite modern hygiene and treatment regimens, it has been estimated that there are around 22 million cases of typhoid fever annually [23]. Also, the increasing spread and frequency of multiresistance to antibiotics in *S.* Typhi is becoming alarming [24].

Serovar Typhimurium (*S.* Typhimurium) in turn causes a more localized, nontyphoidal and usually self-healing inflammatory intestinal infection in humans. This serovar, being more promiscuous in terms of host range, is capable of causing disease in various animals, including mice. In mice, the infection is invasive and resembles typhoid fever [9, 21, 25]. To cause disease in mice, *S.* Typhimurium has to survive in macrophages [26], being a cell type highly proficient in generating ROS and RNS. *S.* Typhimurium is in addition genetically tractable and infects and replicates in professional phagocytic cells. These details have put *S.* Typhimurium in a key position for sorting out facets of bacterial intracellular parasitism, and factors that adapt bacteria to ROS and RNS. The picture that emerges from such studies reveals an image of salmonella being equipped with an array of enzymes and reducing compounds aimed at detoxifying ROS and repairing ROS-induced damage.

S. Typhimurium is closely related to *Escherichia coli* and consequently shares with *E. coli* a relatively large conserved core genome coding for "house-keeping" functions. In addition, *S.* Typhimurium*,* as *S.* Typhi*,* is equipped with numerous virulence genes often contained in smaller or larger horizontally acquired genetic elements named salmonella pathogenicity islands (SPI). SPI1 and SPI2, respectively, play a key role in allowing salmonella to invade the intestinal epithelium and to replicate in professional phagocytic cells [27–29]. SPI1 and SPI2 each code for a type III protein secretion system used for translocating so-called effector proteins into the host cell upon bacteria-host contact. The effector proteins as a rule have very specific functions that interfere with central host cell activities, including actin polymerization, signal transduction, and vesicular trafficking [27–29]. Many serovars, including *S.* Typhimurium, also possess a virulence plasmid characterized by the *spv* virulence genes coding for SPI2 effector proteins [29, 30]. Selected SPI1 and SPI2 effector proteins, as well as the invasion

process itself, will also affect the inflammatory response and consequently ROS and RNS production.

In contrast to *E. coli*, *S.* Typhimurium lacks a capsule, while *S.* Typhi may express the Vi capsular antigen. However, salmonella expresses a lipopolysaccharide with an O antigen; the latter is an important virulence determinant in protection against complement opsonization and phagocytosis [31]. However, the lipid A portion of the LPS molecule is also an activator of innate immunity responses, including induction of iNOS expression [32] and enforcement of the oxidative burst [33]. In addition, salmonella is capable of expressing multiple adhesins that in concert contribute to infection of the mouse [34] and to biofilm formation that adds to the persistent carriage state [35, 36].

Salmonella and ROS

Be it the typhoidal or the nontyphoidal form of salmonellosis, already at the intestinal epithelium *S. enterica* is recognized by pattern recognition molecules and confronted with professional phagocytic cells, eventually resulting in exposure to ROS and RNS [8, 21]. Both ROS and RNS create a central barrier against salmonellosis in the mouse model. Mice not capable of producing an oxidative burst (*phox*–/– mice) quickly succumb to challenge doses of *S.* Typhimurium otherwise coped with by corresponding *phox*+/+ mice [9]. Likewise, humans suffering from chronic granulomatous disease due to a lack of functional phagocyte oxidase show increased sensitivity to invasive, sometimes unorthodox, forms of salmonellosis caused by nontyphoidal servoriants [37–39].

Detoxification of ROS

The superoxide anion generated by the phagocyte NADPH oxidase is charged at neutral pH and thus does not readily diffuse through lipid membranes [40]. However, as such, superoxide could still cause oxidative damage in the periplasmic space [41]. In response, salmonella has periplasmic superoxide dismutases capable of degrading superoxide [42]. Of these, the periplasmic SodCI is needed for virulence of *S.* Typhimurium in *phox*-proficient mice [43–45] but not in *phox*-deficient mice, further pointing to the role of superoxide in restricting proliferation of *S.* Typhimurium [43]. The importance of SodCI in this context is also highlighted by the fact that the corresponding gene (*sodCI*) becomes upregulated even when

S. Typhimurium replicates in nonactivated murine monocytic cells [45, 46] and the fact that *sodCI* is comprised of the salmonella PhoP/PhoQ virulence regulon [47] that also includes the SPI1, SPI2, and the *spv* genes.

S. Typhimurim replicates in an endosomal compartment that is estimated to become moderately low in pH [46, 48]. At these acidities the superoxide anion may become protonated and the superoxide start to diffuse into the bacterial cytoplasm [40]. To cope with protonated superoxide diffusing through the cytoplasmic membrane, the bacterium also codes for 2 cytoplasmic superoxide dismutases, i.e., SodA and SodB. An *sodA* mutant shows moderately decreased survival in murine monocyte-like cells and a slight attenuation in mice [49].

During in vitro oxidative or bile stress, *S.* Typhimurium upregulates the *sitABCD* and *mntH* manganese transport systems [50]. This likely escalates import of Mn^{++} to suppor SodA (an enzyme needing Mn^{++}), leading to an accompanying enhanced superoxide degradation. Indeed, Mn^{++} uptake promotes *S.* Typhimurim survival in the inflamed gut in a mouse model for enterocolitis [51].

Hydrogen peroxide produced by the superoxide dismutases, and any hydrogen peroxide in the close vicinity of salmonella, diffuses more readily through lipid bilayers and is possibly even transported though aquaporins into the cytosol [52]. Nevertheless, this ROS species is met by an array of cytoplasmic enzymes degrading hydrogen peroxide to water and molecular oxygen. These enzymes include 3 catalases (i.e., KatE, KatG, and KatN), and 3 peroxidases (i.e., AhpC, Tpx, and TsaA) [53, 54], yet only an *S.* Typhimurium mutant simultaneously lacking all 3 catalases as well as *ahpC* and *tsaA* has shown hydrogen peroxide sensitization and a replication defect in mice, bone marrow-derived murine macrophages, and murine monocytes like RAW264.7 cells [53]. However, genetic complementation with *katG* or *tsaA* alone restored hydrogen peroxide tolerance and replication in murine RAW264.7 monocytic cells. This points to a high degree of redundancy with regard to the capacity to degrade hydrogen peroxide [55].

While not essential for *S.* Typhimurium intracellular replication, Tpx alone does promote hydrogen peroxide tolerance and increases the intracellular replication propensity in phagocytes [54]. However, this contribution of Tpx to intracellular replication was seen only in IFN-γ activated cells. The use of a Phox inhibitor abrogated the need for Tpx for intracellular replication, indeed pointing a role of Tpx in protecting against ROS.

A twist to detoxification of hydrogen peroxide comes from the observation that the ABC-type efflux pump

MacAB adds to hydrogen peroxide tolerance in *S*. Typhimurium and promotes intracellular replication in murine monocyte like J774 cells [56]. Also, MacAB promotes replication of *S*. Typhimurium in the liver of infected mice. In J774 cells not capable of mounting a respiratory burst, MacAB does not add to intracellular fitness. At first glance one would expect the efflux pump to export hydrogen peroxide from the bacterial cytoplasm. However, intriguingly, a *macAB* mutant revealed a markedly reduced capacity to degrade hydrogen peroxide in vitro, implicating a role of MacAB in degradation rather than in efflux of hydrogen peroxide.

Thiol Chemistry

The periplasmic space poses a special interest with regard to salmonella oxidative stress tolerance, as protein disulfide formation of gram-negative bacteria is conducted in this compartment. This is achieved with the aid of Dsb proteins using Cys-X-X-Cys motifs that undergo oxidation-reduction cycles in forming and breaking disulfide bonds [57, 58]. In this way DsbA acts as a somewhat unspecific oxidoreductase, primarily creating disulfide bonds, DsbB and DsbD act as cytoplasmic membrane electron donors, and DsbC conducts "proofreading" of disulfide bond formation. In addition, the *S*. Typhimurium chromosome codes for the DsbL and DsbI proteins, and these are paralogues for, respectively, DsbA and DsbB [59]. Furthermore, *S*. Typhimurium also codes for the ScsABCD proteins containing Cys-X-X-Cys motifs and for the SrgA disulfide oxidoreductase. ScsB is a homologue of DsbD and has the capacity to reduce ScsC [60], while SrgA assists in formation of the plasmid-encoded Pef-fimbriae [61]. The *S*. Typhimurium periplasmic disulfide oxidases DsbA and SrgA also participate in assembly of the virulence-associated SPI2 protein secretion system [62], while motility relies on DsbA [63].

In the case of oxidative stress one could expect the occurrence of nonenzymatically oxidized thiols and concomitantly an increase in wrongly matched disulfide bridges in periplasmic proteins. As the disulfide oxidases act through disulfide bond formation, the SPI2, Pef, and flagellar supramolecules could be indirect targets of an oxidative attack, e.g., through effects on DsbA and SrgA. Also, exposure to peptidoglycan recognition proteins generates thiol stress in *S*. Typhimurium, contributing to the afore mentioned antibacterial effect of these proteins [19].

That said, surprisingly, mutational inactivation of DsbC in *S*. Typhimurium does not come with major in vitro sensitization to oxidative substances or NO donors [64]. Also, a *dsbC* deletion mutant does not exhibit any apparent attenuation in virulence in BALB/c mice. This could be explained by the presence of the several additional proteins mentioned above that could, or do, take part in disulfide bond formation in the periplasm. Thus, there seems to be redundancy in *S*. Typhimurium with regard to periplasmic (oxido)reductases. That said, when the *scsABCD* genes were deleted in a *dsbC* proficient background, the mutant not only remained virulent but also showed enhanced replication in murine monocyte-like RAW264.7 cells [65]. A rational explanation for this would be that the Dsb system(s) supporting SPI2 assembly competes with the Scs system for redox equivalents, thus contributing to a more efficient SPI2 activity in the absence of the Scs system. Still, *S*. Typhimurium lacking ScsB becomes sensitized to copper chloride [65]. That copper chloride acts as a disulfide catalyst in vitro [66], and de facto conducts disulfide formation of periplasmic proteins in *E. coli* [67], points to a role of the Scs system in restoring wrong disulfide formation upon oxidative stress.

Apart from housing catalases and peroxidases, the bacterial cytoplasm includes the highly reducing enzyme thioredoxin 1 (TrxA). TrxA also operates through a Cys-X-X-Cys motif and assists the Dsb system and ribonucleotide reductase [68]. Still, a *trxA* mutant of *S*. Typhimurium mutant did not reveal any obvious in vitro sensitization to oxidative compounds or NO donors [64]. Even in a very poor medium the tolerance for NO donors was the same for the wild type and a *trxA* mutant. This might appear somewhat surprising as ribonucleotide reductase generates dideoxynucleotides through a tyrosine-associated radical mechanism inhibited by NO [69, 70]. However, the *trxA* the mutant showed a severe replication defect in cultured phagocytic cells and mice due to an inability to translocate virulence-associated SPI2 effector proteins [64].

In part the apparent redundancy of cytoplasmic catalases, peroxidases, and dismutases could be explained by the strong reductant glutathione (contained in mM concentrations in a reduced form in the cytoplasm). Glutathione acts through oxidation of its own thiol group to form an oxidized dimer. Genetic depletion of glutathione synthesis in *S*. Typhimurium caused marked in vitro sensitization to paraquat and hydrogen peroxide but only when the bacteria were grown in medium mimicking the intravacuolar compartment for salmonella (low pH, poor

in nutrients and magnesium [64]). At first glance one would expect the cytoplasm to be a niche protected by glutathione from ROS. However, *E. coli* also possesses a CydDC transport system that shuffles glutathione into the periplasmic space [71], implying that glutathione may add to oxidoprotection in the periplasm as well. *S.* Typhimurium contains the *cyd* homologues, which become upregulated under oxidative stress, albeit not as strongly as many canonical oxidoprotectant genes [50].

Like ROS, the reducing gas hydrogen sulfide (H_2S) is commonly present in biotic habitats, and it is also produced by many bacterial species. Hydrogen sulfide has also been proposed to act as a general protectant against various classes of antibiotics mechanistically through oxidoprotective mechanisms [72]. A classical diagnostic parameter for *S.* Typhimurium in the microbiological laboratory is its ability to produce large amounts of hydrogen sulfide [73]. Thus, it would not be surprising if *S.* Typhimurium also applies hydrogen sulfide when coping with ROS. Indeed, the genes for thiosulfate reductase, CysIJ involved in hydrogen sulfide production, become upregulated upon in vitro hypochlorite stress [74] and peroxide stress [50], as well as under in vitro conditions that mimic the environment of the salmonella-containing intracellular vacuole [50].

Damage Repair

Two additional classes of reductases have been identified as adding tolerance to ROS and virulence in *S.* Typhimurium through reduction of oxidized sulfur groups. In this biotin sulfoxide reductase converts biothine sulfoxide back to biothine. Biotin sulfoxide reductase adds to hydrogen peroxide tolerance in *S.* Typhimurium, as well as to the ability to replicate in murine monocytic cells [75]. Likewise, methionine sulfoxide reductase MsrA, that generates methionine from methionine sulfoxide, improves hydrogen peroxide tolerance and increases the fitness of *S.* Typhimurium in IFN-γ-activated murine monocytic cells, as well as in mice [76].

Apart from oxidizing sulfhydryl and sulfur groups, ROS also causes the conversion of aspartate to *iso*-aspartate, a reaction reverted by isoaspartate methyl transferase. In *S.* Typhimurium the gene for this enzyme, i.e., *pimt*, is needed for full tolerance to ROS and for growth in IFN-γ-activated peritoneal murine macrophages [77]. Inhibition of Phox by apocynin decreased the need for *pimt*. This would support the notion that the role of *pimt* indeed originates from coping with Phox-generated ROS.

The effect of ROS on nucleic acids is dual. First, nucleic acids act as a target for ROS-induced damage causing strand breaks, mutagenesis, and modification of nucleotide bases [3, 78, 79]. Indeed, components of the DNA repair machinery, such as *recA*, *lexA*, and *sulA* appear to be of high importance for virulence and ROS tolerance in *S.* Typhimurium [79–82]. In *S.* Typhimurium, the lack of RecA, a protein needed for DNA repair and induction of the SOS response, results in substantial sensitization to ROS and a strong attenuation with regard to virulence in mice [80]. Likewise, bacterial RNA has been implicated as a main target for ROS [78]. In this, the ribonuclease polynucleotide phosphorylase has been proposed to protect *E. coli* against hydrogen peroxide through degradation of oxidized RNA. However, an *S.* Typhiumurium mutant lacking functional polynucleotide phosphorylase does not reveal increased sensitization to hydrogen peroxide (unpubl. res.). However, the *S.* Typhimurium polynucleotide phosphorylase participates in the regulation of SPI1 and SPI2 gene expression [83] and could thus indirectly contribute to ROS adaptation.

A second line of effects caused by ROS on DNA is at the gene regulatory level. *E. coli* possess redox-sensing transcriptional regulators, such as OxyR, SoxR, and SoxS, that regulate, for example, the expression of the *ahpC*, *ahpF*, *katG*, and *sodA* genes, as well as genes involved in DNA repair and methionine synthesis [84]. In this, OxyR acts through the formation of an intermolecular Cys-X-X-Cys cysteine bridge to sense oxidation. Transcriptomic profiling of S. Typhimurium shows that basically the homologues of the whole *E. coli* OxyR/SoxR/SoxS regulon, in terms of upregulated genes, are also induced in *S.* Typhimurium upon oxidative shock [50]. Nevertheless, genetic depletion of OxyR in *S.* Typhimurium does not affect the ability of the mutant to survive in neutrophils [85], while the Sox regulon seems to be required for *S.* Typhimurium tolerance to paraquat [82].

In *E. coli, dps* codes for a DNA-binding ferritin-like protein that becomes highly abundant in the stationary phase [86]. Dps binds DNA and confers increased resistance to oxidative stress [87–89], possibly by physically protecting DNA from ROS-induced damage. Upon oxidative and nitrosative stress *S.* Typhimurium strongly upregulates the expression of its *dps* [50]. The gene is also needed for *S.* Typhimurium survival in primary murine macrophages and for virulence in mice [89]. Apart from a possible role in directly protecting DNA, Dps could also add to oxidoprotection through scavenging of iron, thus preventing Fenton reactions.

Hypochlorite is an ROS produced by neutrophils in response to infection, and a compound found in many disinfectants. *E. coli* possess a transcriptional regulator, i.e., YjiE, that responds to hypochlorite and confers hypochlorite tolerance [90]. A homologue for *yjiE* is present in *S.* Typhimurium and *yijE* expression becomes activated upon oxidative stress [50], implying that *S.* Typhimurium may also have a hypochlorite-protection regulon.

Nitric Oxide

As implied above, when salmonella encounters professional phagocytes these cells may become activated and start producing RNS (NO) with the aid of iNOS and ROS present. Like ROS, RNS may cause a multitude of damages, such as protein nitrosylation or formation of metal complexes. Mice lacking iNOS become sensitized to *S.* Typhimurium, albeit not to the same extent as *phox*(–/–) mice [9]. Still, murine macrophages and dendritic cells infected with *S.* Typhimurium and simultaneously treated with iNOS inhibitors lose their ability to control intracellular replication of the bacteria [46, 91]. All of this implies that salmonella should have measures to cope with RNS. Indeed, an *S.* Typhimurium mutant lacking *sodCI*, apart from being sensitized to superoxide, becomes highly sensitized to a mixture of superoxide and an NO donor [42]. A rational explanation for this could be that SodCI prevents the formation of peroxynitrite through degradation of superoxide radicals.

Flavohemoglobins belongs to the hemoglobin superfamily and consists of 2 domains, i.e., a FAD-binding oxidoreductase domain and a heme-containing domain. *E. coli* and *S.* Typhimurium both encode for the flavohemoglobin Hmp. It protects against NO by oxidizing NO into nitrate under aerobic conditions [92–95]. Hmp is also needed for the survival of *S.* Typhimurium in human macrophages [94] and the *hmp* gene becomes induced as *S.* Typhimurium replicates in murine macrophages [46].

E. coli furthermore possesses the NorRVW (previously YgaAKD) NO protection system [96]. This system converts NO into nitrous oxide (N_2O) at lower oxygen tensions. The corresponding genes are present in *S.* Typhimurium, with *norV* and *norW* being strongly induced for expression upon in vitro-induced NO stress [50].

Intriguingly, De Groote et al. [97] noted that one could increase NO tolerance in *S.* Typhimurium by deleting the genes for the stress tolerance sigma factor RpoS or the Dpp dipeptide transport system. The mechanism(s) behind these observations remains to be sorted out, but one

possible explanation is that the sigma factor and peptide transport systems would strongly distort fitness if nitrosylated by RNS. While RpoS activates *katE* expression, expression of *rpoS* itself is not induced upon either peroxide or NO stress [50].

Preventing ROS and RNS Production

While invasion of *S.* Typhimurium of epithelial cells results in an inflammatory response trough SPI effector proteins, i.e., LPS and flagellin, selected effector proteins also possess a potential anti-inflammatory activity. For example, the SPI1 effector protein AvrA (from avirulence protein A) prevents NF-kB nuclear translocation [98]. This would prevent the expression of several cytokines and iNOS in phagocytes. Likewise, several SPI2 effector proteins, such as SpvC, SseL, and SspH1, pose functions potentially downregulating inflammatory activation of the infected host cells [29], with, for example, SpvC dephosphorylating phosphor-threonine from phospho-ERK [99]. *S.* Typhi, the human-adapted serovar that causes typhoid fever, codes for a cytolethal-distending toxin (CDT) not present in *S.* Typhimurium. The toxin acts by being a nuclease. Surprisingly though, when the *S.* Typhi CDT is implanted in *S.* Typhimurium the intestinal inflammation score is highly reduced in orally infected mice [100]. Expression of CDT also causes downregulation of the expression of cytokines and iNOS in intestinal epithelial tissue. This implies that salmonella through its CDT dampen intestinal inflammation and likely thereby exposure of salmonella to ROS and RNS.

It is also possible to isolate phagocyte-adapted mutants of *S.* Typhimurium that downregulate NO production with an accompanying increased intracellular growth capacity [91]. The mechanism(s) remains to be solved, but for all such mutants isolated the effect appeared to be on iNOS activity rather than on iNOS expression. As iNOS relies on L-arginine for NO production, one tentative mechanism would be to, one way or another, deplete the phagocyte from L-arginine.

Biofilm Formation

Biofilms can be defined as microbial multicellular communities embedded in a macromolecular mass produced by a single or different microbial species therein [36, 101, 102]. The benefits of living in such communities are probably many, but from a perspective of clinical mi-

Fig. 1. Summary of strategies by which *S.* Typhimurium copes with oxidative stress: (1) degradation of ROS before they act on target molecules, as exemplified by superoxide dismutases (SodA and SodCI), catalase (KatE), and thiol peroxidase (Tpx); (2) balancing periplasmic disulfide bond formation, as exemplified by the thioredoxin, Dsb, and Scs systems; (3) transport of cofactors for detoxifying enzymes; (4) redoxsensing gene regulatory systems lodging on, e.g., superoxide disumutase genes; (5) direct protective or iron-scavenging systems (Hmp) or (6) repairing enzymes (methionine sulfoxide reductase MsrA); (7) application of secreted effector proteins such as AvrA with the potential to downregulate an inflammatory response; and (8) production and transport of small-molecular reducing compounds to appropriate locations.
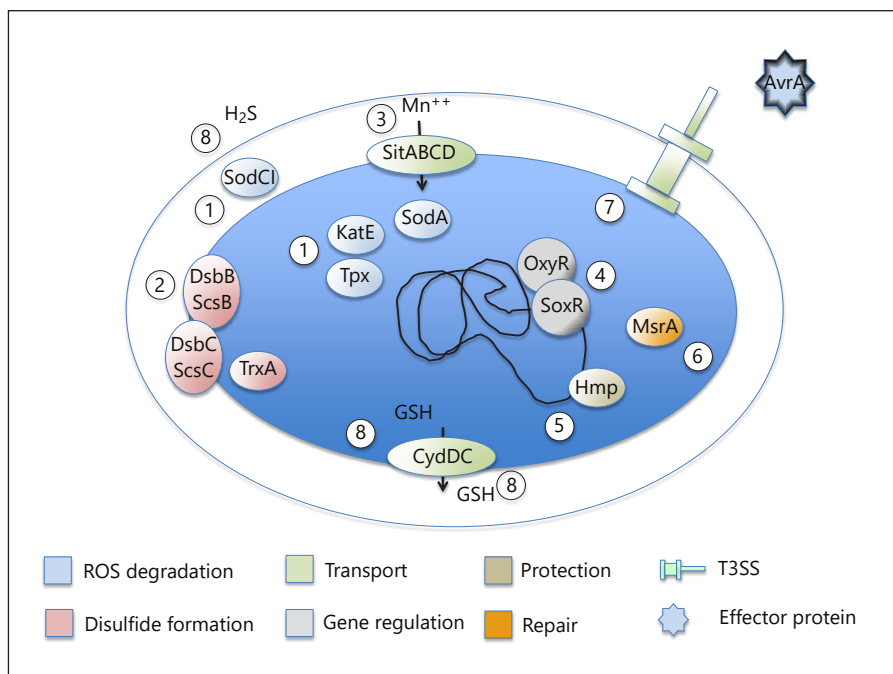
crobiology one could imagine the following: adherence to abiotic and biotic surfaces, the ability to resist host immune defense, and an increased tolerance to antimicrobial compounds [103].

Salmonella is capable of forming biofilms on gall bladder stones, which is believed to promote establishment of carriage [35], despite constant exposure to bile and hence ROS stress as stipulated above. *S.* Typhimurium biofilm formation is characterized by a decreased expression of motility (planktonic mode), accompanied by the expression biofilm extracellular matrix components such as amyloid curli fimbriae and cellulose fibers (sessile mode) [36]. In conjunction with the second messenger cyclic-di-GMP-associated gene regulatory network, the CsgD gene regulator plays a key role in regulation of biofilm formation. Several observations connect biofilm formation with oxidative stress. As *S.* Typhimurium enters biofilm formation, apart from upregulating *csgD* it also upregulates genes associated with ROS stress [104]. In parallel, hypochlorite stress induces in *S.* Typhimurium a transcriptomic signature suggestive of an adaptation towards sessile biofilm formation [74]. Growth of *S.* Typhimurium on the oxidative surface of a redox-active conducting polymer also enhanced biofilm formation [105]. This could all imply that, at least under some redox stress conditions, *S.* Typhimurium prefers to shift from the planktonic to the sessile biofilm mode to adapt to ROS. This

would be consistent with the induction of biofilm formation in, for example, *Campylobacter jejuni* [106] and *Staphylococcus aureus* [107] as a response to oxidative stress.

Biofilm formation also connects to redox in the sense that deleting either *dsbA* or *dsbB* results in CsgD-dependent upregulation of biofilm formation in *S.* Typhimurium [108]. Furthermore, this upregulation relies on the c-di-GMP phosphodiesterase STM3615, thus linking periplasmic protein thiol chemistry to biofilm formation and c-di-GMP.

Making Use of ROS

By expressing pathogen-associated microbial patterns, such as flagellin and LPS, *S.* Typhimurium is clearly itself responsible for evoking inflammation [32]. Intriguingly, salmonella evidently makes use of an inflammatory response with an accompanying ROS production. For one thing, intestinal inflammation induced by *S.* Typhimurium infection skews the intestinal microbial flora in mice, such that it favors salmonella colonization [109]. This competitive advantage may be further potentiated in that the inflammation-associated ROS production creates tetrathionate, a sulfur oxanion, in the intestine from preexisting sulfur compounds [110]. Tetrathionate in turn can

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be used as a respiratory electron sink by *S.* Typhimurium [110, 111], thus in principle allowing S. Typhimurium to take advantage of the oxidative potential of ROS for its own respiration in an otherwise anaerobic or hypoxic environment.

S. Typhimurium can also sense oxidative/nitrosative stress with the aim of inducing virulence. The virulence-associated SPI2 response regulator SsrB becomes S-nitrosylated at Cys 203 upon NO stress [112]. While it did not affect the expression of selected SPI2 genes, a mutant with an *ssrRB* allele lacking the critical SsrB Cys residue showed decreased fitness in a murine infection model. This could suggest that *ssrB* regulates virulence factor genes outside of SPI2 but in response to RNS.

Intriguingly, *S.* Typhimurium seems to be able to sense the neurotransmitter adrenaline followed by induction of sodA [113]. The connection may appear farfetched, but adrenaline, like selected other neurotransmitters, participates in regulation of inflammatory responses [114]. Thus, while acting more to dampen inflammation, adrenaline could still be used by *S.* Typhimurium to sense an inflammatory environment, potentially being enriched in ROS.

Thus, ROS production clearly is a double-edged sword from the perspective of both salmonella and its host. ROS is obviously needed for protection against salmonellosis, as evidenced by infection experiments with *phox*–/– mice and by the increased prevalence of invasive salmonellosis in patients suffering from chronic granulomatous disease. *S.* Typhimurium in turn translocates SPI1 and SPI2 effector proteins during the infection which have the potential to dampen an inflammatory response [96, 97], possibly to prevent too early a clearance, yet host-derived ROS and RNS adds to its in vivo replication potential. Furthermore, the so-called "typhoid" CDT-like toxin dampened intestinal inflammation in a mouse infection model [100]. Thus, salmonella seems to try to balance the host response (Fig. 1) to allow a certain degree of ROS response to increase its fitness and to allow induction of virulence genes.

Disclosure Statement

The author declares no commercial interest or conflict of interest.

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