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REVIEW ARTICLE

Bacterial Virulence Factors: Secreted for Survival

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Abstract Virulence is described as an ability of an organism to infect the host and cause a disease. Virulence factors are the molecules that assist the bacterium colonize the host at the cellular level. These factors are either secretory, membrane associated or cytosolic in nature. The cytosolic factors facilitate the bacterium to undergo quick adaptive—metabolic, physiological and morphological shifts. The membrane associated virulence factors aid the bacterium in adhesion and evasion of the host cell. The secretory factors are important components of bacterial armoury which help the bacterium wade through the innate and adaptive immune response mounted within the host. In extracellular pathogens, the secretory virulence factors act synergistically to kill the host cells. In this review, we revisit the role of some of the secreted virulence factors of two human pathogens: *Mycobacterium tuberculosis*—an intracellular pathogen and *Bacillus anthracis*—an extracellular pathogen. The advances in research on the role of secretory factors of these pathogens during infection are discussed.

Keywords *Mycobacterium* · Virulence · *Bacillus* · Anthrax · Tuberculosis · Phosphatases

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Mycobacterium tuberculosis

Mycobacterium tuberculosis (Mtb) is the causative agent of the disease "Tuberculosis" and is thought to infect 33% of world's human population. Mtb, an obligate aerobic intracellular pathogen, gains entry into its host through the respiratory route via aerosols generated by an infected individual. Once inside the pulmonary alveoli, the pathogen is entrapped by alveolar macrophages, neutrophils, dendritic cells (DCs) or even epithelial cells [1, 2]. Various ligands present on the mycobacterial cell surface such as phosphatidylinositol mannoside (PIM), HSP70, 19 kDa lipoprotein, lipoarabinomannan (LAM) are recognised by phagocytic receptors and/or specific pattern recognition receptors (PRRs) present at the cell membrane of macrophages or DCs [3]. Uptake through some of the receptors is thought to be favourable for the pathogen's survival [4]. Within the phagosomes, Mtb generates a complex response and undergoes substantial metabolic shift to adapt in the hostile environment [5]. The success of Mtb lies in its potential to arrest the phagosome maturation thereby creating a safe haven for its survival. The host on the other hand actively initiates signaling cascades in the infected cells [6]. The DCs and macrophages reach the lymph nodes and the infection is thus communicated to the lymphoid cells. This marks the beginning of the adaptive immunity. After the adaptive immunity sets in, the bacterial growth is restricted and the lymphoid cells along with the myeloid cells gather to form a condensed structure called granuloma [7]. Granulomas, initially thought to be a strategy adopted by the host to contain infection, are now proposed to act as structures assisting the bacterium in successful dissemination [8]. The ability of Mtb to utilise granulomas for dissemination and also its ability to survive in primary innate immune cells, the macrophages, shows how successfully

the pathogen has co-evolved with humans. Mtb has garnered multiple ways to manipulate the host's primary defence system for its survival and dissemination. To accomplish this feat, Mtb utilises plethora of virulence factors which act at various levels during the pathogen's life cycle [9]. Some of the identified virulence factors assist the bacterium adapt physiologically and metabolically in the hostile environment, still others are secreted and carry out a range of biological and immunological modulations within the host [9].

The modulations carried out in the host are executed most efficiently and rapidly through disruptions in the host signaling network [6]. Signaling molecules such as bacterial kinases and phosphatases are most proficient in carrying out this desired role. In recent years, secretory phosphatases belonging to distinct families; (1) Protein-tyrosine phosphatases (MptpA, MptpB), (2) Lipid phosphatases (SapM), and recently identified, (3) Phosphoserine phosphatase SerB2, have been identified as key molecules in the virulence of Mtb. The secreted tyrosine-phosphatase MptpA has been shown to interfere with phagosome acidification by inhibiting the trafficking of vacuolar-ATPase to phagosome [10, 11]. MptpB, another secreted tyrosine-phosphatase has been shown to subvert the host immunity [12]. These phosphatases have been the subject of some elegant reviews in recent years [13–15]. In this review we revisit the role of the other two secreted phosphatases, SapM (an acid phosphatase) and SerB2 (a phosphoserine phosphatase), in the adaptation of the pathogen within the host.

SapM

The secreted proteins are the primary component of Mtb's armoury against its host. In 1998, Raynaud et al. [16] carried out a comprehensive research in which they identified enzymatic activities in the secreted polypeptides from various mycobacterial species. The culture filtrate protein fractions from both pathogenic as well as non-pathogenic strains were shown to possess an acid phosphatase activity, one of the 24 identified enzymatic activities [16]. Acid phosphatase is the class of enzymes that catalyses the breakdown of phosphomonoesters (phosphoenolpyruvate, glycerophosphate, guanosine triphosphate, NADPH, phosphotyrosine, trehalose-6-phosphate etc.) under acidic conditions [17]. Two years later, the acid phosphatase from the culture filtrate protein fraction responsible for the observed activity was identified as a 28-kDa protein [18]. N-terminal sequencing unveiled the presence of a typical prokaryotic signal sequence and hence it was proposed that the protein would be secreted through the sec-dependent mechanism. The protein was thus named secreted acid phosphatase of Mtb, SapM. Sequence analysis of SapM however showed no homology with prokaryotic acid phosphatases, instead SapM was shown to have a high homology with several acid phosphatases of fungal origin [18].

Research carried out on acid phosphatases from various other intracellular pathogens indicated towards its role in intracellular survival by virtue of its ability to subdue the respiratory burst of activated human neutrophils [19–21]. In 2005, Vergne et al. showed that *M. tuberculosis* var. *bovis* Bacillus Calmette–Guérin (BCG) SapM could abrogate phagosomal maturation by reducing the concentration of membrane-bound lipid phosphatidylinositol 3-phosphate (PI3P) from the phagosomal membranes [22]. Rab5, a Rab GTPase, recruits phosphatidylinositol 3-kinase (PI3K), which in turn phosphorylates Phosphatidylinositol (PI) converting it into PI3P (Fig. 1a). PI3P influences the function and cellular localization of various effector proteins with PI3P binding domains (e.g., EEA1). The recruited proteins in turn mediate phagosomal maturation into phago-lysosome [23]. To persist in the phagosomes, Mtb should be able to maintain a PI3P free status. SapM was shown to dephosphorylate PI3P resulting in the reduction of its concentration on the phagosomal membrane and hence the stalling of phagosomal biogenesis (Fig. 1a) [22]. Festjens et al. [24] however, proposed an alternate mode of action for Bacillus Calmette-Guerin (BCG) SapM. While trying to study the vaccine potential of BCG mutant deficient in SapM, they showed that SapM mutation had no effect on the lysosome fusion with BCG containing phagosomes. They further showed that the mutation had no apparent effect on reactive oxygen species (ROS) production, autophagy or the activation of iNKT cells. Instead, the BCG SapM mutant was shown to induce the secretion of TNF-α, IL-6 and IL-1β, from infected Bone marrow-derived dendritic cells (BM-DCs) which in turn resulted in enhanced migration and activation of DCs in draining lymph nodes. They concluded that SapM mutant could enhance presentation of antigen in a pro-inflammatory environment. This study implicated that Mtb SapM would somehow block the activation and recruitment of DCs thus helping the bacteria lower down the severity of adaptive immune response. The studies which followed [25, 26] however, unequivocally indicated toward a role of Mtb SapM in phagosome maturation arrest thereby substantiating the results obtained by Vergne et al. [22]. In an attempt to create Mtb derived vaccine strain with a potential to undergo phagosome lysosome fusion, Saikolappan et al. [25] generated a double knock out (DKO) strain, by deleting the genes *fbpA* and *sapM* in Mtb (Δ*fbpA*Δ*sapM*). They had previously reported that the disruption of *fbpA* (Ag85A) gene from Mtb resulted in the attenuation of the mutant within the macrophages [27]. Using THP1 macrophages they demonstrated that the DKO mutant showed higher attenuation as the mutant pathogen

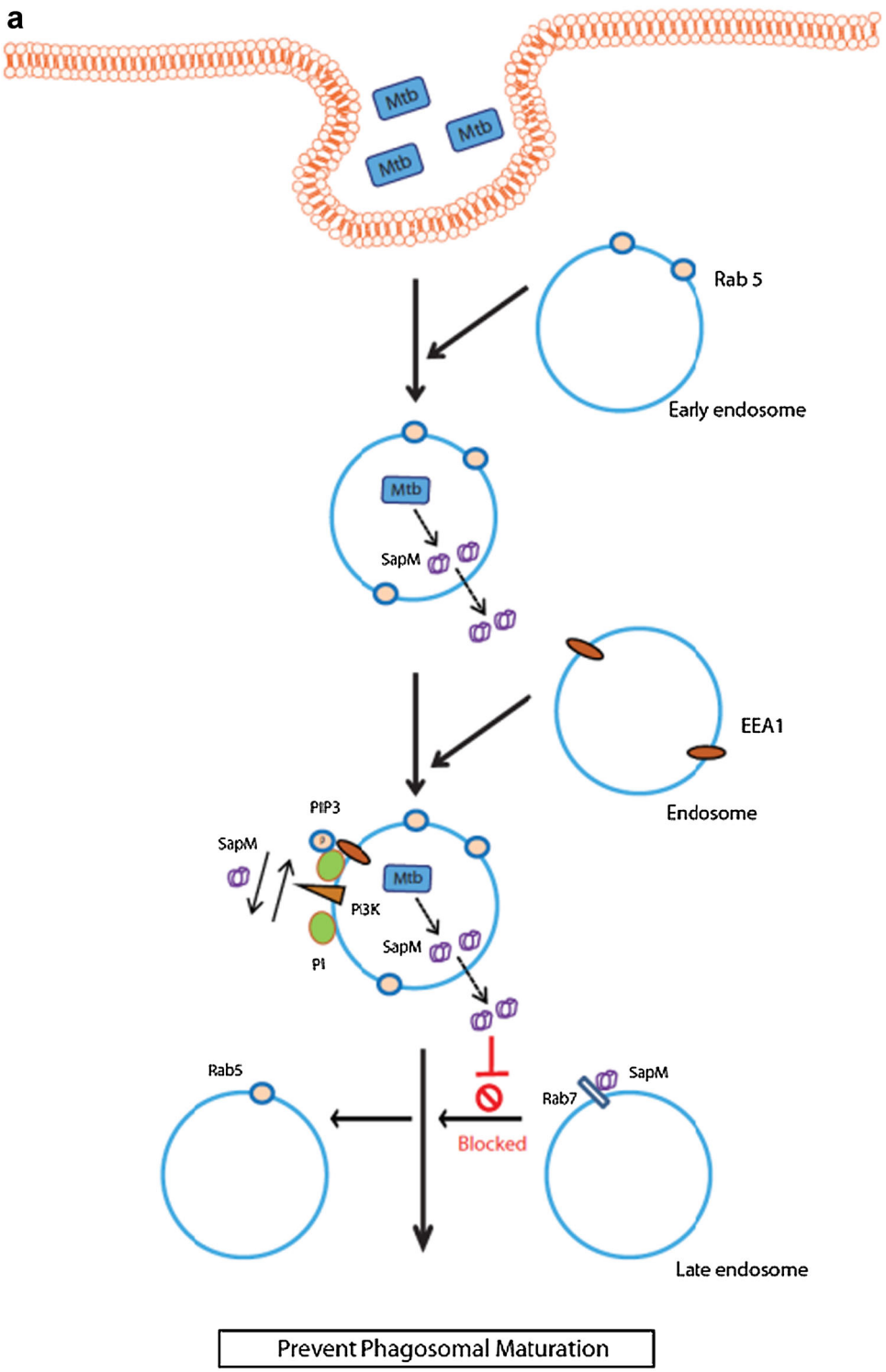
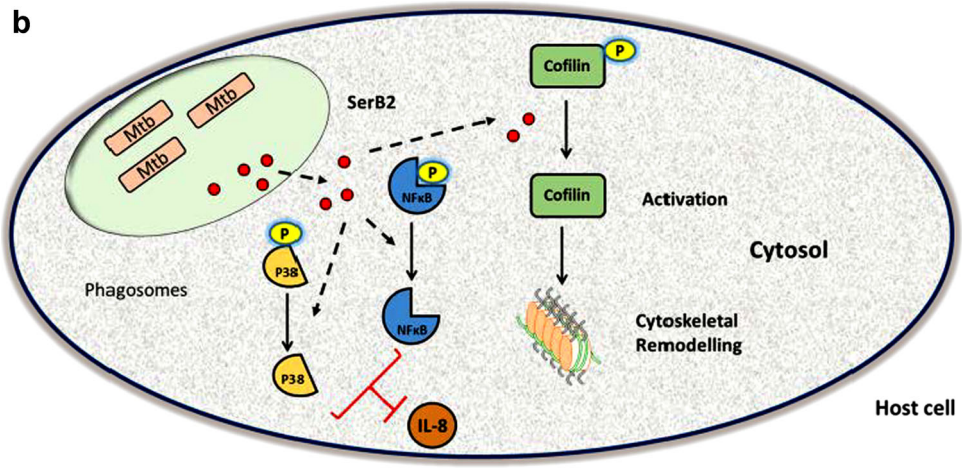


Fig. 1 Secretory phosphatase-SapM and SerB2 of *Mycobacterium tuberculosis*. **a** SapM is secreted by Mtb and enters host cells by an unknown mechanism. SapM dephosphorylates PI3P causing reduction of PI3P concentration on the phagosomal membrane. This in turn inhibits the recruitment of EEA1 protein, preventing phagosomal maturation. Additionally, SapM has been shown to block Rab7 through its CT domain, resulting in the inhibition of phagosomal maturation into a phagolysosome. **b** SerB2 dephosphorylates MAPK-p38 and NFκB p65 to suppress IL-8 synthesis in macrophages. SerB2 also dephosphorylates cofilin leading to its activation causing cytoskeleton remodelling

was unable to inhibit phagosome lysosome fusion in the infected host cell. Subsequently the DKO mutant was highly immunogenic, owing to its higher antigen presentation [25]. The DKO mutant was also found to be susceptible to oxidants, indicating towards a role of SapM in repressing the oxidative burst as seen for acid phosphatases from other intracellular pathogens [19–21]. Similar results using Mtb*ΔsapM* mutant were obtained by Puri et al. [26]. The study was taken a step further and conducted in an animal model and guinea pigs infected with Mtb*ΔsapM* were shown to have an enhanced survival in comparison to Mtb infected animals. It was observed that SapM was

Fig. 1 continued



indispensable for Mtb growth and that the infection with Mtb*ΔsapM* mutant caused a significant reduction in pathological damage in the host [26]. The same group carried out another study to understand the effect of vaccination with the triple knockout mutant Mtb*ΔmptpAΔmptpBΔsapM* (Mtb*Δmms*) versus BCG on the survival and pathogenesis of Mtb [28]. They reported that the vaccination with Mtb*Δmms* mutant restricted the growth of Mtb at primary site of infection quite in comparison to BCG. However, the vaccination with the mutant could not control the hematogenous spread when compared to BCG. The authors indicated the need to improvise the mutant to provide a superior protection and a control over hematogenous spread. Finally, in a recent report, Hu et al. [29] showed that SapM exposure to macrophages resulted in the accumulation of autophagosomes. SapM was shown to block Rab GTPase, Rab7, through its CT domain (C-terminal domain). Notably, the phagosomal maturation is dependent on the replacement of Rab5 with Rab7 which in turn directs the maturation of phagosome to phagolysosome.

In summary, SapM has been identified as a secreted virulence factor required for the arrest of phagosomal maturation. Various vaccine strains of Mtb and BCG with *sapM* deletion have been generated. However, how SapM flips to the cytosolic side of the phagosomal membrane to carry out the observed activities remains unknown.

SerB2

SerB2 is the most recent addition in the repertoire of Mtb's secreted virulence factors [30]. Initially characterized as an enzyme involved in the dephosphorylation of *O*-phospho-L-serine to L-serine [31], MtSerB2 has been recently shown to be secreted into the cytoplasm of infected THP1 cells and is hypothesized to help the bacterium in immune invasion and evasion [30]. SerB2 belongs to the haloacid dehalogenase (HAD) class of enzymes. The enzyme class HAD shows a ubiquitous presence across all three super kingdoms of life and are usually involved in metabolic processes [32]. However, there have been reports where some HADs were shown to exhibit divergent function such as transcription regulation or cytoskeleton remodelling [33–35].

In the first report on MtSerB2, Arora et al. [31] biochemically characterized the enzyme and showed that MtSerB2 displayed a preference toward *O*-phospho-L-serine over *O*-phospho-L-threonine. Through high throughput screening using 2300 compounds, they identified Clorobiocin and Rosalinine as the specific inhibitors against MtSerB2. The inhibitors displayed bactericidal activity against Mtb infected macrophages. However, since Clorobiocin targets other mycobacterial factors also, the authors suggested a need to develop structural analogs of the identified inhibitors with MtSerB2 specific intracellular activity. Around the same time, Yadav et al. [36] published another report on MtSerB2 characterization. MtSerB2 contains two small molecule binding ACT domains (Aspartate kinase, Chorismate mutase, and TyrA protein regulatory domain) at its N-terminus and a classical phosphoserine phosphatase domain (PSP) toward the C-terminal end. Yadav et al. showed that both PSP and ACT domains are required for the optimal phosphatase activity of MtSerB2. The ACT domain was identified as the regulatory domain and interaction of various amino acid/ligand was shown to induce an increase or decrease or no effect on MtSerB2 activity. This study, however, added another dimension to the role of MtSerB2 in Mtb and the authors showed that the exogenously added MtSerB2 resulted in microtubule re-arrangement in THP-1 cells. This experiment indicated that MtSerB2 acted in a similar capacity as SerB653 from *Porphyromonas gingivalis* (*P. gingivalis*), previously shown to induce microtubule re-arrangement in HIGK cells (human immortalized gingival

keratinocytes) [35, 37, 38]. Recently, the same group published a follow-up study and showed that MtSerB2 was secreted in the cytoplasm of infected THP1 macrophages [30]. MtSerB2 was shown to interact with various host proteins involved in actin remodelling (actin, cofilin, a/b tubulin, etc.) and was capable of dephosphorylating cofilin leading to its activation (activated cofilin causes actin depolymerisation) (Fig. 1b). MtSerB2 was also shown to regulate the expression of genes that are involved in the actin dynamics. Additionally, through in vitro pull down assays it was shown that MtSerB2 interacts with HSP90, HSP70 and HSP27, hence blocking apoptosis. Exogenously added MtSerB2 was shown to suppress IL-8 expression in THP1 macrophages, primarily due to its ability to dephosphorylate MAPK-p38 and NFκB p65 (Fig. 1b). These experiments were primarily based on research published on SerB653 from *P. gingivalis* and effectively reproduced what was known for SerB653 [39, 40]. Finally, they identified a drug Clofazimine {evaluated for XDR (Extremely drug resistant) and MDR (Multiple drug resistant) TB treatment} which inhibits MtSerB2 activity and reverse the observed interactions.

In summary, the research conducted on SerB2 has helped in demonstrating that apart from its conventional role of a metabolic enzyme, MtSerB2 has acquired a new function wherein it is secreted in the host and interacts with host factors to assist the bacterium in invasion and evasion leading to its survival and pathogenesis.

Bacillus anthracis

Anthrax is caused by a Gram positive, rod shaped and endospore forming bacteria, *Bacillus anthracis*. The pathogenicity of *Bacillus anthracis* is governed by two large extrachromosomal plasmids namely pXO1 and pXO2. The plasmid pXO1 encodes protective antigen, lethal factor, edema factor and anthrax toxin activator A (AtxA), a central regulator for toxin synthesis [41]. These secreted toxin units enable the pathogen to establish a systemic infection in short duration of time. On the other hand, plasmid pXO2 encodes for the proteins involved in capsule synthesis. The capsule is made up of poly-γ-D-glutamic acid, that inhibits the bacterial phagocytosis during infection and is weakly immunogenic in nature [42]. Another major virulence factor is anthrolysin O, which is one of the pore forming toxins and disrupts the membrane integrity of host cells by forming homogenous pores [43]. The systemic infection of anthrax is majorly established by lethal toxin (protective antigen + lethal factor) and edema toxin (protective antigen + edema factor). The carboxyl terminal of protective antigen interacts with the cell membrane receptors like Capillary Morphogenesis Gene 2 (CMG2) and Tumor Endothelial Marker 8 (TEM8) and facilitates the entry of lethal factor and edema factor into the cytoplasm of host cells [44]. Protective antigen, being the central molecule in the anthrax toxin pathogenesis, is an exciting target molecule for the development of advance therapeutics against anthrax. Multifaceted approach has been employed to target the protective antigen of the anthrax toxin, for example, use of non-functional protective antigen (dominant negative) and CMG2 or TEM8 receptor decoys [45, 46]. Recent research exploring the pathogen's strategies to modulate the host signaling via its secreted virulence factors like lethal toxin, edema toxin and anthrolysin O is quite fascinating and is discussed in the following sections.

Lethal Toxin

Lethal factor is a Zn^{2+} metal dependent endoprotease and the active site residues (HEXXH) interact with the divalent metal ion (Zn^{2+}) to attain the active protein conformation [47]. Lethal factor targets the members of MAP Kinase Kinase (MAPKK) family of MAP cascade, which includes MEK 1–4, 6, and 7 [48]. The cleavage of N-terminus of MAPKK members results in a diverse set of phenotypes in the intoxicated host cells. Lethal toxin mediated cleavage of MAPKK causes decrease in the phosphorylation status of host 'Heat Shock Protein 27' (HSP27), which is reported to be essential for maintaining the permeability across endothelial cell lining (Fig. 2a). Activity of HSP27 is governed through the phosphorylation and therefore the membrane permeability is found to be compromised in anthrax infections [49]. Systemically released lethal toxin is also reported to reduce the phosphorylation level of translational proteins for examples eIF4B, eIF4E and rps6. The changes in active translational factors causes decreased translation of Hypoxia-Induced Factor-1α (HIF1 α), a central protein involved in the host cell response against hypoxic condition. Severely decreased expression of HIF-1α could lead to pathological conditions in host like angiogenesis and metastasis [50]. The implications of N-terminus cleavage of MAPKK family members have been found to be diverse. Apart from changing the protein expression or phosphorylation status of physiologically important host proteins, lethal toxin disrupts the host response to anthrax in additional ways too. For example, inflammasomes, which are the multimeric protein complexes involved in the identification of pathogen associated molecular patterns in cytosol and directs the activity of caspase 1 enzyme to counter bacterial infections. Lethal toxin cleaves the N-terminus of NOD like receptor protein 1 (Nlrp1), which results in the activation of caspase-1 in the host cell, which in turn causes N-terminus cleavage of pre- IL 1β and pre-IL 18 (Fig. 2a). These two inflammatory

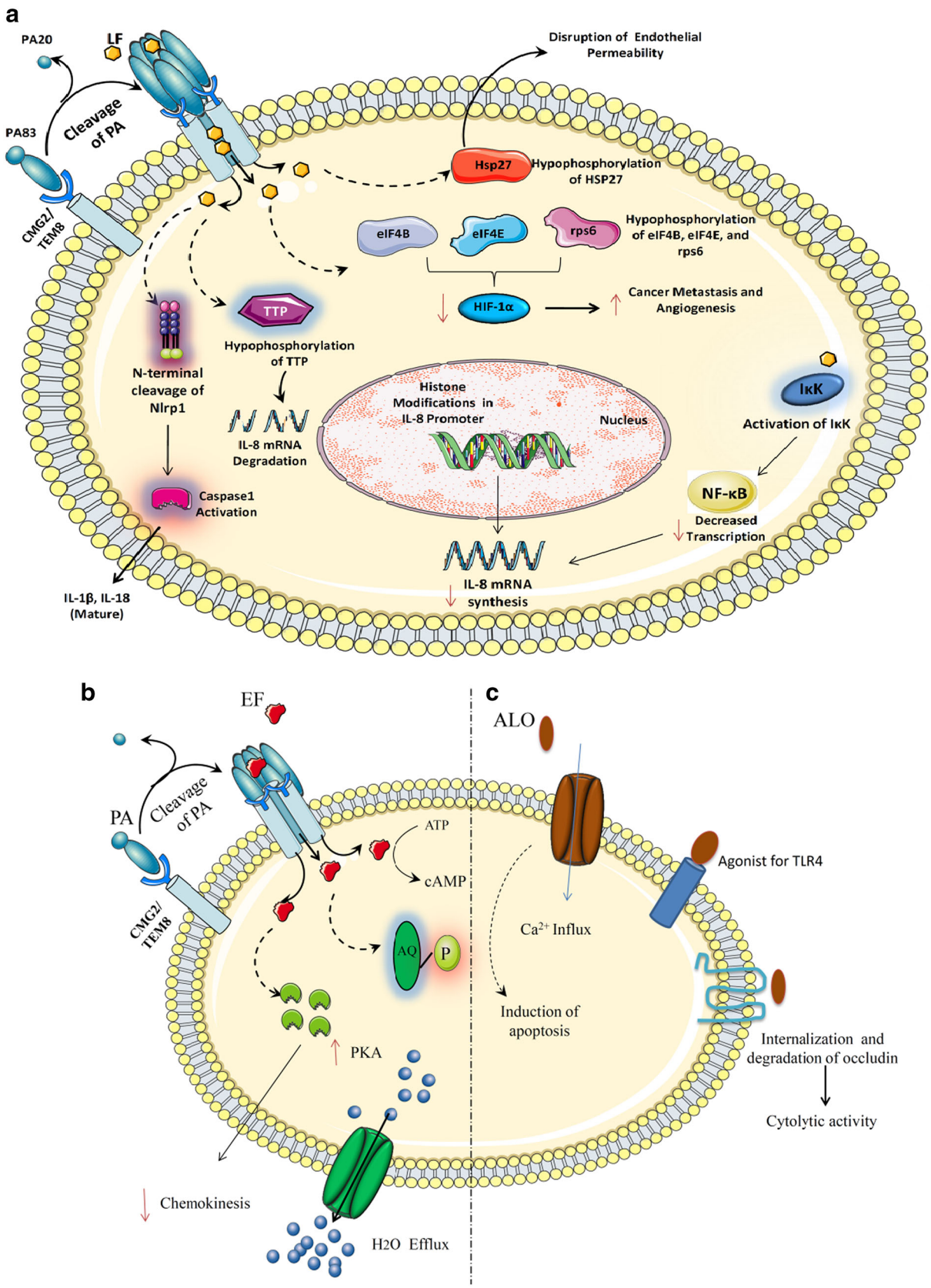


Fig. 2 Schematic diagram explaining the physiological imbalances observed in the host cells by anthrax major secreted virulence factors, which are lethal toxin (LT), edema toxin (ET), and anthrolysin O (AL)

cytokines are well known to initiate the primary innate immune response upon infection [51, 52].

Bacterial infections are accompanied by the recruitment of polymorphonuclear cells at the site of pathogen's invasion. This crucial primary response against pathogen is however hindered by the lethal toxin moiety of anthrax toxin system. Cytokine IL-8 is an important chemoattractant, which is sensed by the neutrophils and is responsible for the migration of neutrophils to the site of injury. Lethal toxin is reported to cause modifications in the histone epigenetics of IL-8 promoter (Fig. 2a), which causes lesser binding of NF-κB transcription factor, hence causing reduced translational rates of IL-8 [53]. However, the exact molecular pathways through which these modifications take place are yet to be elucidated. On the other hand, lethal toxin intoxication to the host cells also results in enhanced IL-8 degradation through the hypophosphorylation of TTP, a RNA binding protein involved in the degradation of IL-8. It has been reported that localization of TTP has been increased to the processing bodies during anthrax infection. Furthermore, the number of processing bodies has been found to be increased, implying higher level of degradation in lethal toxin intoxicated cells [54]. In the host system, lethal toxin also acts by decreasing the NF-κB transcription via activation of IκK kinase (transcription inhibitor) (Fig. 2a). However, the expression of NF-κB regulated genes are over-expressed as well as under-expressed depending on the expression levels of other transcriptional regulators involved [55]. During infection, lethal factor is expressed in higher amounts and imparts higher toxicity to the intoxicated cells when compared to the edema factor. The edema toxin mediated toxicity and known mechanisms are discussed in the next section.

Edema Toxin

Edema toxin is another virulence factor of *Bacillus anthracis*. Edema factor acts as adenylyl cyclase and is dependent on calmodulin and calcium ions for its activity. Upon infection, edema toxin gains entry into the host cell cytoplasm through PA translocation channel and converts the cellular pool of adenosine triphosphate (ATP) into 3′, 5′ cyclic adenosine monophosphate (cAMP), a secondary messenger crucial in diverse cellular processes [56]. Elevated and persistent level of cAMP causes loss of water from cells into the intercellular milieu (Fig. 2b). It has been recently elucidated that the presence of edema toxin alters the phosphorylation status of water channels across cell membrane, aquaporin-2, hence activating them for increased water efflux, specifically in the collecting ducts of kidney [57]. Excess water retention is the hallmark action of edema toxin; apart from this, edema toxin is also reported to significantly alter the host defence mechanism. Upon bacterial infection, polymorphonuclear cells play crucial role in trapping the bacteria and decrease the pathogen load from the host. The pathogen entry site is recognized by the release of cytokines like Interferon γ, which is followed by the recruitment of leukocytes at the entry site. Edema toxin inhibits the chemokinesis of the polymorphonuclear cells, resulting in decreased accumulation of cells at the site of pathogen's invasion. The observed phenotype is accompanied with increased phosphorylation of Protein Kinase A (Fig. 2b); however the physiological impact of which, is yet to be elucidated [58].

Anthrolysin O

Anthrolysin O is another potent virulence factor secreted by the virulent strain of *Bacillus anthracis*, and belongs to the Cholesterol Dependent Cytolysins (CDC) class of cytolysins. Anthrolysin O forms pores in the host cell membrane and disrupt the membrane integrity causing host cell death. Formation of pores results in a diverse set of downstream signaling, for example increased influx of calcium ion in the intoxicated cells [59]. Introduction of anthrolysin O causes disruption of C2BBE monolayer through altering the arrangement of Occludin, an integral membrane protein involved in maintaining the cellular tight junction [60] (Fig. 2c). This cytolytic activity of anthrolysin O is extended to cell types like human monocytes, human monocyte derived macrophages, neutrophils, erythrocyte and lymphocytes. The haemolytic activity of anthrolysin O is hindered by the presence of free cholesterol [61]. *Bacillus anthracis* is among the few Gram-positive bacteria, which produces membrane vesicles. Membrane vesicles derived from *Bacillus anthracis* is reported to contain the anthrolysin O apart from lethal toxin/edema toxin, indicating the physiological role of anthrolysin O during anthrax infections [62]. Bishop and colleague have studied the effect of recombinant purified anthrolysin O on the integrity of gut epithelial barrier in human C2BBE and ex vivo mouse intestinal segments. It has been observed that disruption of C2BBE monolayer leads to higher mobility of bacteria across the basolateral transwell. *Bacillus anthracis* strain secreting anthrolysin O can cross monolayer more readily as compared to the anthrolysin deficient bacterial strain [63]. The human alpha defensins are the host counter measures to check the hemolysis caused by anthrolysin O [64]. Monoclonal antibodies administration against anthrolysin O causes increased survival of intoxicated animal mice (BALB/c) model system [65]. Also, it has been reported that the function of anthrolysin O and three phospholipases are dispensable in *Bacillus anthracis*. Only, deletions of all four genes can cause attenuation of the *Bacillus anthracis*

strain in in vitro and in vivo infection models [66]. Interestingly, anthrolysin O is also known to act as an agonist for Toll Like Receptor 4, which usually gets activated by lipopolysaccharides secreted by gram negative bacteria. Furthermore, in combination with lethal factor, anthrolysin O is known to induce apoptosis in intoxicated cells (Fig. 2c) [67]. The structure, function and physiological relevance of anthrolysin O has been well elucidated during the last decade, however there are no studies investigating the specificity of various drug molecules to inhibit the anthrolysin O, till now.

Conclusion and Future Directions

The success of a pathogen to establish an infection in the host relies on its ability to counter the continuous onslaught mounted by the host to clear off the infection [6]. The mediators of virulence in the bacterial system are often the moonlighting proteins, which are the primary weapons for the host targeting and thus needs our immediate attention. Pathogens, whether intracellular or extracellular in nature, extensively utilize the secreted proteins to modulate the central pathways of host's signaling machinery [9, 45]. In this review, we have discussed some important virulence factors of two human pathogens, Mtb—the causative agent of the disease tuberculosis and *B. anthracis*—the causative agent of the disease anthrax. Since, the secreted factors can be effectively targeted by therapeutic molecules or vaccines, an in-depth understanding of their role in virulence will assist in identification of the 'right factors' which are most crucial for the pathogen's survival. Apart from secreted proteins, pathogens also secrete small molecule or quorum sensing molecules, which are important for the pathogen to establish infection and the role of these molecules has been well studied in pathogenic as well as non-pathogenic bacteria [68–73]. This review enhance our understanding about the crucial roles played by the secreted virulence factor of Mtb and *B. anthracis*. Focussing on the secretory factors that are fundamental to the infectious process will help us to generate a repertoire of effective small molecule inhibitors or prophylactic vaccines that may be advantageous in combating the bacterial attack.

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References

1. Dorhoi A, Kaufmann SH (2011) Versatile myeloid cell subsets contribute to tuberculosis-associated inflammation. Eur J Immunol 45:2191–2202. doi:10.1002/eji.201545493
2. Lerner TR, Borel S, Gutierrez MG (2015) The innate immune response in human tuberculosis. Cell Microbiol 17:1277–1285. doi:10.1111/cmi.12480
3. Dorhoi A, Reece ST, Kaufmann SH (2011) For better or for worse: the immune response against *Mycobacterium tuberculosis* balances pathology and protection. Immunol Rev 240:235–251. doi:10.1111/j.1600-065X.2010.00994.x
4. Delogu G, Provvedi R, Sali M, Manganelli R (2015) *Mycobacterium tuberculosis* virulence: insights and impact on vaccine development. Future Microbiol 10:1177–1194. doi:10.2217/fmb.15.26
5. Rohde KH, Veiga DF, Caldwell S, Balázsi G, Russell DG (2012) Linking the transcriptional profiles and the physiological states of *Mycobacterium tuberculosis* during an extended intracellular infection. PLoS Pathog 8:e1002769. doi:10.1371/journal.ppat.1002769
6. Koul A, Herget T, Klebl B, Ullrich A (2004) Interplay between mycobacteria and host signalling pathways. Nat Rev Microbiol 2:189–202. doi:10.1038/nrmicro840
7. Guirado E, Schlesinger LS (2013) Modeling the *Mycobacterium tuberculosis* granuloma—the critical battlefield in host immunity and disease. Front Immunol 4:98. doi:10.3389/fimmu.2013.00098
8. Davis JM, Ramakrishnan L (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 9:37–49. doi:10.1016/j.cell.2008.11.014
9. Forrellad MA, Klepp LI, Gioffré A, Sabio y García J, Morbidoni HR, de la Paz Santangelo M, Cataldi AA, Bigi F (2013) Virulence factors of the *Mycobacterium tuberculosis* complex. Virulence 4:3–66. doi:10.4161/viru.22329
10. Koul A, Choidas A, Treder M, Tyagi AK, Drlica K, Singh Y, Ullrich A (2000) Cloning and characterization of secretory tyrosine phosphatases of *Mycobacterium tuberculosis*. J Bacteriol 182:5425–5432. doi:10.1128/JB.182.19.5425-5432.2000
11. Wong D, Bach H, Sun J, Hmama Z, Av-Gay Y (2011) *Mycobacterium tuberculosis* protein tyrosine phosphatase (PtpA) excludes host vacuolar-H + -ATPase to inhibit phagosome acidification. Proc Natl Acad Sci USA 108:19371–19376. doi:10.1073/pnas.1109201108
12. Zhou B, He Y, Zhang X, Xu J, Luo Y, Wang Y, Franzblau SG, Yang Z, Chan RJ, Liu Y, Zheng J, Zhang ZY (2010) Targeting mycobacterium protein tyrosine phosphatase B for antituberculosis agents. Proc Natl Acad Sci USA 107:4573–4578. doi:10.1073/pnas.0909133107
13. Wong D, Chao JD, Av-Gay Y (2013) *Mycobacterium tuberculosis*-secreted phosphatases: from pathogenesis to targets for TB drug development. Trends Microbiol 21:100–109. doi:10.1016/j.tim.2012.09.002
14. Fanzani L, Porta F, Meneghetti F, Villa S, Gelain A, Lucarelli AP, Parisini E (2015) *Mycobacterium tuberculosis* low molecular weight phosphatases (MPtpA and MPtpB): From biological insight to inhibitors. Curr Med Chem 22:3110–3132. doi:10.3390/md12052953
15. Sajid A, Arora G, Singhal A, Kalia VC, Singh Y (2015) Protein phosphatases of pathogenic bacteria: role in physiology and virulence. Annu Rev Microbiol 69:527–547. doi:10.1146/annurev-micro-020415-111342
16. Raynaud C, Etienne C, Peyron P, Laneelle MA, Daffe M (1998) Extracellular enzyme activities potentially involved in the pathogenicity of *Mycobacterium tuberculosis*. Microbiology 144:577–587. doi:10.1099/00221287-144-2-577

17. Bull H, Murray PG, Thomas D, Fraser AM, Nelson PN (2002) Acid phosphatases. Mol Pathol 55:65–72. doi:10.1136/mp.55.2.65
18. Saleh MT, Belisle JT (2000) Secretion of an acid phosphatase (SapM) by *Mycobacterium tuberculosis* that is similar to eukaryotic acid phosphatases. J Bacteriol 182:6850–6853. doi:10.1128/JB.182.23.6850-6853.2000
19. Remaley AT, Kuhns DB, Basford RE, Glew RH, Kaplan SS (1984) Leishmanial phosphatase blocks neutrophil O-2 production. J Biol Chem 259:11173–11175
20. Reilly TJ, Baron GS, Nano FE, Kuhlenschmidt MS (1996) Characterization and sequencing of a respiratory burst-inhibiting acid phosphatase from *Francisella stularensis*. J Biol Chem 271:10973–10983. doi:10.1074/jbc.271.18.10973
21. Saha AK, Dowling JN, Lamarco KL, Das S, Remaley AT, Olomu N, Pope MT, Glew RH (1985) Properties of an acid phosphatase from *Legionella micdadei* which blocks superoxide anion production by human neutrophils. Arch Biochem Biophys 243:150–160. doi:10.1016/0003-9861(85)90783-0
22. Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V (2005) Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 102:4033–4038. doi:10.1073/pnas.0409716102
23. Russell DG (2011) *Mycobacterium tuberculosis* and the intimate discourse of a chronic infection. Immunol Rev 240:252–268. doi:10.1111/j.1600-065X.2010.00984.x
24. Festjens N, Bogaert P, Batni A, Houthuys E, Plets E, Vanderschaeghe D, Laukens B, Asselbergh B, Parthoens E, De Rycke R, Willart MA, Jacques P, Elewaut D, Brouckaert P, Lambrecht BN, Huygen K, Callewaert N (2011) Disruption of the SapM locus in *Mycobacterium bovis* BCG improves its protective efficacy as a vaccine against *M. tuberculosis*. EMBO Mol Med 3:222–234. doi:10.1002/emmm.201000125
25. Saikolappan S, Estrella J, Sasindran SJ, Khan A, Armitige LY, Jagannath C, Dhandayuthapani S (2012) The fbpA/sapM double knock out strain of *Mycobacterium tuberculosis* is highly attenuated and immunogenic in macrophages. PLoS ONE 7:e36198. doi:10.1371/journal.pone.0036198
26. Puri RV, Reddy PV, Tyagi AK (2013) Secreted acid phosphatase (SapM) of *Mycobacterium tuberculosis* is indispensable for arresting phagosomal maturation and growth of the pathogen in guinea pig tissues. PLoS ONE 8:e70514. doi:10.1371/journal.pone
27. Armitige LY, Jagannath C, Wanger AR, Norris SJ (2000) Disruption of the genes encoding antigen 85A and antigen 85B of *Mycobacterium tuberculosis* H37Rv: effect on growth in culture and in macrophages. Infect Immun 68:767–778. doi:10.1128/IAI.68.2.767-778.2000
28. Chauhan P, Reddy PV, Singh R, Jaisinghani N, Gandotra S, Tyagi AK (2013) Secretory phosphatases deficient mutant of *Mycobacterium tuberculosis* imparts protection at the primary site of infection in guinea pigs. PLoS ONE 8:e77930. doi:10.1371/journal.pone.0077930
29. Hu D, Wu J, Wang W, Mu M, Zhao R, Xu X, Chen Z, Xiao J, Hu F, Yang Y, Zhang R (2015) Autophagy regulation revealed by SapM-induced block of autophagosome-lysosome fusion via binding RAB7. Biochem Biophys Res Commun 461:401–407. doi:10.1016/j.bbrc.2015.04.051
30. Shree S, Singh AK, Saxena R, Kumar H, Agarwal A, Sharma VK, Srivastava K, Srivastava KK, Sanyal S, Ramachandran R (2016) The *M. tuberculosis* HAD phosphatase(Rv3042c) interacts with host proteins and is inhibited by Clofazimine. Cell Mol Life Sci 73:3401–3417. doi:10.1007/s00018-016-2177-2
31. Arora G, Tiwari P, Mandal RS, Gupta A, Sharma D, Saha S, Singh R (2014) High throughput screen identifies small molecule inhibitors specific for *Mycobacterium tuberculosis* phosphoserine phosphatase. J Biol Chem 289:25149–25165. doi:10.1074/jbc.M114.597682
32. Seifried A, Schultz J, Gohla A (2013) Human HAD phosphatases: structure, mechanism, and roles in health and disease. FEBS J 280:549–571. doi:10.1111/j.1742-4658.2012.08633.x
33. Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA, Selengut JD, Parlikar BE, Rebay I (2003) The transcription factor eyes absent is a protein tyrosine phosphatase. Nature 426:299–302. doi:10.1038/nature02097
34. Gohla A, Birkenfeld J, Bokoch GM (2005) Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. Nat Cell Biol 7:21–29. doi:10.1038/ncb1201
35. Tribble GD, Mao S, James CE, Lamont RJ (2006) A *Porphyromonas gingivalis* haloacid dehalogenase family phosphatase interacts with human phosphoproteins and is important for invasion. Proc Natl Acad Sci USA 103:11027–11032. doi:10.1073/pnas.0509813103
36. Yadav GP, Shree S, Maurya R, Rai N, Singh DK, Srivastava KK, Ramachandran R (2014) Characterization of *M. tuberculosis* SerB2, an essential HAD-family phosphatase, reveals novel properties. PLoS ONE 9:e115409. doi:10.1371/journal.pone.0115409
37. Hasegawa Y, Tribble GD, Baker HV, Mans JJ, Handfield M, Lamont RJ (2008) Role of *Porphyromonas gingivalis* SerB in gingival epithelial cell cytoskeletal remodeling and cytokine production. Infect Immun 76:2420–2427. doi:10.1128/IAI.00156-08
38. Bainbridge B, Verma RK, Eastman C, Yehia B, Rivera M, Moffatt C, Bhattacharyya I, Lamont RJ, Kesavalu L (2010) Role of *Porphyromonas gingivalis* phosphoserine phosphatase enzyme SerB in inflammation, immune response, and induction of alveolar bone resorption in rats. Infect Immun 78:4560–4569. doi:10.1128/IAI.00703-10
39. Moffatt CE, Inaba H, Hirano T, Lamont RJ (2012) *Porphyromonas gingivalis* SerB-mediated dephosphorylation of host cell cofilin modulates invasion efficiency. Cell Microbiol 14:577–588. doi:10.1111/j.1462-5822.2011.01743.x
40. Takeuchi H, Hirano T, Whitmore SE, Morisaki I, Amano A, Lamont RJ (2013) The serine phosphatase SerB of *Porphyromonas gingivalis* suppresses IL-8 production by dephosphorylation of NF-kappaBRelA/p65. PLoS Pathog 9:e1003326. doi:10.1371/journal.ppat.1003326
41. Hammerstrom TG, Roh JH, Nikonowicz EP, Koehler TM (2011) *Bacillus anthracis* virulence regulator AtxA: oligomeric state, function and CO(2) signalling. Mol Microbiol 82:634–647. doi:10.1111/j.1365-2958.2011.07843.x
42. Makino S, Wataraj M, Cheun HI, Shirahata T, Uchida I (2002) Effect of the lower molecular capsule released from the cell surface of *Bacillus anthracis* on the pathogenesis of anthrax. J Infect Dis 186:227–233. doi:10.1086/341299
43. Shannon JG, Ross CL, Koehler TM, Rest RF (2003) Characterization of anthrolysin O, the *Bacillus anthracis* cholesterol-dependent cytolysin. Infect Immun 71:3183–3189. doi:10.1128/IAI.71.6.3183-3189.2003
44. Singh Y, Klimpel KR, Quinn CP, Chaudhary VK, Leppla SH (1991) The carboxyl terminal end of protective antigen is required for receptor binding and anthrax toxin activity. J Biol Chem 266:15493–15497
45. Dhasmana N, Singh LK, Bhaduri A, Misra R, Singh Y (2014) Recent developments in anti-dotes against anthrax. Recent Pat Antiinfect Drug Discov 9:83–96. doi:10.2174/1574891X09666140830213925
46. Singh Y, Khanna H, Chopra AP, Mehra V (2001) A dominant negative mutant of *Bacillus anthracis* protective antigen inhibits anthrax toxin action *in vivo*. J Biol Chem 276:22090–22094. doi:10.1074/jbc.M010222200

47. Quinn CP, Singh Y, Klimpel KR, Leppla SH (1991) Functional mapping of anthrax toxin lethal factor by in-frame insertion mutagenesis. J Biol Chem 266:20124–20130
48. Duesbery NS, Webb CP, Leppla SH, Gordon VM, Klimpel KR, Copeland TD, Ahn NG, Oskarsson MK, Fukasawa K, Paull KD, Vande Woude GF (1998) Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. Science 280:734–737. doi:10.1126/science.280.5364.734
49. Liu T, Milia E, Warburton RR, Hill NS, Gaestel M, Kayyali US (2012) Anthrax lethal toxin disrupts the endothelial permeability barrier through blocking p38 signaling. J Cell Physiol 227:1438–1445. doi:10.1002/jcp.22859
50. Ouyang W, Torigoe C, Fang H, Xie T, Frucht DM (2014) Anthrax lethal toxin inhibits translation of hypoxia-inducible factor 1α and causes decreased tolerance to hypoxic stress. J Biol Chem 289:4180–4190. doi:10.1074/jbc.M113.530006
51. Levinsohn JL, Newman ZL, Hellmich KA, Fattah R, Getz MA, Liu S, Sastalla I, Leppla SH, Moayeri M (2012) Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog 8:e1002638. doi:10.1371/journal.ppat.1002638
52. Chavarria-Smith J, Vance RE (2013) Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor. PLoS Pathog 9:e1003452. doi:10.1371/journal.ppat.1003452
53. Raymond B, Batsche E, Boutillon F, Wu YZ, Leduc D, Balloy V, Raoust E, Muchardt C, Goossens PL, Touqui L (2009) Anthrax lethal toxin impairs IL-8 expression in epithelial cells through inhibition of histone H3 modification. PLoS Pathog 5:e1000359. doi:10.1371/journal.ppat.1000359
54. Chow EM, Batty S, Mogridge J (2010) Anthrax lethal toxin promotes dephosphorylation of TTP and formation of processing bodies. Cell Microbiol 12:557–568. doi:10.1111/j.1462-5822.2009.01418.x
55. Warfel JM, D'Agnillo F (2009) Anthrax lethal toxin enhances IkappaB kinase activation and differentially regulates pro-inflammatory genes in human endothelium. J Biol Chem 284:25761–25771. doi:10.1074/jbc.M109.036970
56. Leppla SH (1982) Anthrax toxin edema factor: a bacterial adenylate cyclase that increases cyclic AMP concentrations of eukaryotic cells. Proc Natl Acad Sci USA 79:3162–3166
57. Sastalla I, Tang S, Crown D, Liu S, Eckhaus MA, Hewlett IK, Leppla SH, Moayeri M (2012) Anthrax edema toxin impairs clearance in mice. Infect Immun 80:529–538. doi:10.1128/IAI.05947-11
58. Szarowicz SE, During RL, Li W, Quinn CP, Tang WJ, Southwick FS (2009) *Bacillus anthracis* edema toxin impairs neutrophil actin-based motility. Infect Immun 77:2455–2464. doi:10.1128/IAI.00839-08
59. Gekara NO, Westphal K, Ma B, Rohde M, Groebe L, Weiss S (2007) The multiple mechanisms of Ca^{2+} signalling by listeriolysin O, the cholesterol-dependent cytolysin of *Listeria monocytogenes*. Cell Microbiol 9:2008–2021. doi:10.1111/j.1462-5822.2007.00932.x
60. Bourdeau RW, Malito E, Chenal A, Bishop BL, Musch MW, Villereal ML, Chang EB, Mosser EM, Rest RF, Tang WJ (2009) Cellular functions and X-ray structure of anthrolysin O, a cholesterol-dependent cytolysin secreted by *Bacillus anthracis*. J Biol Chem 284:14645–14656. doi:10.1074/jbc.M807631200
61. Mosser EM, Rest RF (2006) The *Bacillus anthracis* cholesterol-dependent cytolysin, Anthrolysin O, kills human neutrophils, monocytes and macrophages. BMC Microbiol 6:56. doi:10.1186/1471-2180-6-56
62. Rivera J, Cordero RJ, Nakouzi AS, Frases S, Nicola A, Casadevall A (2010) *Bacillus anthracis* produces membrane-derived vesicles containing biologically active toxins. Proc Natl Acad Sci USA 107:19002–19007. doi:10.1073/pnas.1008843107
63. Bishop BL, Lodolce JP, Kolodziej LE, Boone DL, Tang WJ (2010) The role of anthrolysin O in gut epithelial barrier disruption during *Bacillus anthracis* infection. Biochem Biophys Res Commun 394:254–259. doi:10.1016/j.bbrc.2010.02.091
64. Lehrer RI, Jung G, Ruchala P, Wang W, Micewicz ED, Waring AJ, Gillespie EJ, Bradley KA, Ratner AJ, Rest RF, Lu W (2009) Human alpha-defensins inhibit hemolysis mediated by cholesterol-dependent cytolysins. Infect Immun 77:4028–4040. doi:10.1128/IAI.00232-09
65. Nakouzi A, Rivera J, Rest RF, Casadevall A (2008) Passive administration of monoclonal antibodies to anthrolysin O prolong survival in mice lethally infected with *Bacillus anthracis*. BMC Microbiol 8:159. doi:10.1186/1471-2180-8-159
66. Heffernan BJ, Thomason B, Herring-Palmer A, Hanna P (2007) *Bacillus anthracis* anthrolysin O and three phospholipases C are functionally redundant in a murine model of inhalation anthrax. FEMS Microbiol Lett 271:98–105. doi:10.1111/j.1574-6968.2007.00713.x
67. Park JM, Ng VH, Maeda S, Rest RF, Karin M (2004) Anthrolysin O and other gram- positive cytolysins are toll-like receptor 4 agonists. J Exp Med 200:1647–1655. doi:10.1084/jem.20041215
68. Koul S, Prakash J, Mishra A, Kalia VC (2016) Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria. Indian J Microbiol 56:1–18. doi:10.1007/s12088-015-0558-0
69. Kalia VC (2013) Quorum sensing inhibitors: An overview. Biotechnol Adv 31:224–245. doi:10.1016/j.biotechadv.2012.10.004
70. Kalia VC, Wood TK, Kumar P (2014) Evolution of resistance to quorum-sensing inhibitors. Microb Ecol 68:13–23. doi:10.1007/s00248-013-0316-y
71. Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140. doi:10.3109/1040841X.2010.532479
72. Kalia VC, Raju SC, Purohit HJ (2011) Genomic analysis reveals versatile organisms for quorum quenching enzymes: Acyl-homoserine lactone-acylase and –lactonase. Open Microbiol J 5:1–13. doi:10.2174/1874285801105010001
73. Kalia VC (2014) Microbes, antimicrobials and resistance: the battle goes on. Indian J Microbiol 54:1–2. doi:10.1007/s12088-013-0443-7