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Anti-bacterial vaccine activities of Zn, ZnONPs and Zn^{2+}-induced activated peptidoglycan autolysins against Gram-positive and Gram negative Bacteria

Review Article

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Abstract

Firstly, against Gram-positive bacteria, adsoption of Zn^{2+} ions to the bacterial cell surface increases cell wall cohesion and favors the projection of elongated SasG away from the cell surface. Zinc importer adcABC of the primary group A streptococcus (GAS) zinc uptake system is composed of a cell surface-exposed zinc-binding protein (adcA), an inner membrane permease (AdcB), and a cytosolic ATPase (AdcC) that provides the energy for zinc import by ATP hydrolysis. Multivalent fusion DNA vaccine against *Brucella abortus* has been constructed that the expression of BAB antigens conjugated to SOD protein can polarize mice immunity to a Th1-type phenotype.

ZnO-NPs are attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. Bacteriolytic activity of ZnO-NPs is associated with the generation of ROS including H_2O_2, OH^-, and O_2^{-2} that ROS have been cell wall damage due to ZnO-localized interaction, enhanced membrane permeability, internalization of Nps due to loss pf proton motive force and uptake of toxic dissolved zinc ions. Released zinc ions from zinc oxide penetrate the bacterial cell wall via diffusion that ZnO-NPs disintegrate the cell membrane and accumulate in the cytoplasm. ZnO-NPs caused significant up-regulation of biosynthesis and degradation.

S.aureus amidase AmiA of PGN autolysin is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated molecule. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophyticus*) is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats. AmiB catalyzes the degradation of PGN in bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell separation and PGN fragments release. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial vaccine activities. Lytic amidase autolysin LytA associates with the cell wall via its zinc-binding motif. The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc-β-(1,4)-MurNAc glycosidic bond of PGN building units. LytC, LytD, and LytF are expressed in the same subpopuration of cells and complete flagellar synthesis.

Secondly, against Gram-negative bacteria, ZnuA is a high affinity acquisition of Zn^{2+} in *E. coli* was demonstrated and shown to occur via the ABC permease, Znu ABC that the Znu permease comprises the SBP ZnuA, and an ABC tranporter. The acquisition of zinc by *P. aeruginosa* PAO_1 reveals a hitherto unrecognized complexity in zinc homeostasis that enables the bacterium to survive under zinc limitation that the mechanisms and pathways uitilyzed by *P. aeruginosa* to survive and promulgate in environments of varying Zn^{2+} abundance, with the findings widely applicable to other

prokaryotic organisms. Recombinant flagella and pili to targeting lipo-polysacharides and O-antigens have shown some promise in a preventing infection that outer membrane protein including OprF and OprI are newer representative of vaccine candidates. Recombinant AfeA expresses abundant epitopes on the bacterial surface and induces protective responses in the mouse pulmonary clearance model following aerosol challenge with *Moraxella catarrhalis.* ETEC is the most common bacerial cause of children's diarrea, in which antigen and antitoxin antibodies that neutralized both toxins that are associated with all cases of ETEC diarrhea, and polypeptide or subunit vaccines have the potential to effectively protect against ETEC diarrhea. Thus, the antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress such as *Campylobacter*.

Amidase gene (AmiB) catalyzes the degradation of PGN in Gram-negative bacteria that the amiB is involved in the separation of daughter cells after cell devision and inactivation of the amiB gene, resulting in a marked increases of sensitivity to oxidative stress and organic acids. AmiC controls cell separation and PGN fragments release. Zinc-dependent endopeptidases are predicted to hydrolyze PGN to facilitate cell growth that zinc avaliability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria. Zinc-regulated peptidase maintains cell wall integrity during immune-mediated nutrient sequestration against *Acinetobacter baumannii*. Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxypeptidase B1 of and its evaluation have been high molecular characterization for TBVs against Malaria eradication. MCPs of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi*. Thus, zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial vaccines. Autolysin mediated bacteriolysis- and zinc dependent lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities. Human PGLYRPs are novel class of recognition and effector molecules with broad Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria.

Keywords

ZnO-NPs, PGN hydrolase and autolysin, Zn^{2+} ions-induced autolysin, Zinc dependent anti-bacterial vaccine, Amidase, ROS

Abbreviations

Aas= autolysin/adhesin of *Staphylococcus saprophyticus, ABC*=ATP-binding cassette, APC=antigen presenting cell, *A. stephensi= Anopheles stephensi, B.abortus= Brucella abortus, B. subtilis= Bacillus subtilis,* CBPs=choline binding proteins, *C. difficile= Clostridium difficile,* CKD=Chronic Kidney Disease, *E. coli=Escherichia coli, E. faecalis=Enterococcus faecalis, E. faecium=Enterococcus faecium,* ETEC= Enterotoxigenic *E.coli, Eps=Zinc dependent endopeptidases,* EVs=extracellular vesicles, *FnBPs*=fibronectin-binding proteins, Gas=group A streptococcus, GelE=zinc metalloprotease, gelatinase, *M. catarrhalis=Moraxella catarrhalis,* MCPs= Metallocarboxy-peptidases, MIBRs=most probable immuno-protective B-cell epitope regions, MRB=multidrug bacteria, *MRSA=methicillin-resistant Staphylococcus aureus,* NAM=N-acetylmuramic acid, NAG=N-acetylglucosamine, *N. meningitidis=Neisseria meningitidis,* ORSs=oral rehydration solutions, ORT=oral rehydration therapy, *P. aeruginosa= Pseudomonas aeruginosa,* PBP2a=penicilline-binding protein2a, PGN=peptidoglycan, PGRPs=peptidoglycan recognition proteins, PSP=plasmid stabilization protein, ROS=reactive oxygen species, Sags=superantigens, SasG=*S. aureus* surface protein, *S.aureus*= *Staphylococcus aureus,* SBP=solute-binding protein, SEB=staphylococcal entoxin serotype B, SOD=superoxide dismutase, *S. pneumoniae=Streptococcus pneumoniae,* TBVs=tranmission-blocking vaccines, VRE=vancomycin-resistant Enterococcus faecium, ZnONPs= Zinc oxide (ZnO) nanoparticles, ZBL=zinc binding lipoprotein, ZnuA=Zinc uptake A. Zur=zinc uptake regulator.

Introduction

Zinc is known to play a central role in the immune system that zinc exert a pharmacological action on immune responses to antigens in the intestine which zinc reduces the antitoxin and enhance the antibacterial responses in serum and oral zinc administration of vaccine has the potential to modify critical immune responses to antigens applied to mucosal surfaces [1]. Zinc is an essential element reuired for hair metabolism and the activation of the immune system that the vaccines induced a stage of zinc deficiency by activating the immune system and

increased the utilization of zinc [2]. The innate immune system uses zinc as an antimicrobial agent and that zinc efflux is an important contributor to such as group A *Streptococcus* (GAS) pathogenesis [3]. Zinc is the second most abundant trace metal with human body 2-3g, 90% in muscle and bone, and 10% other organs include prostate, liver, the gastrointestinal tract, kidney, skin, lung brain, heart, and pancreas in humans which cellular zinc underlies an efficient homeostatic control that avoids accumulation of zinc in excess [4]. The role of zinc in cell death has apoptosis that the influence of zinc on apoptosis is tissue/cell type, zinc concentration, and expression of zinc transporters and zinc-binding proteins. Host zinc homeostasis changes in response to bacterial infections, including production of metal sequestering proteins and bombard-ment of bacteria with toxic level of zinc at host-pathogen interface [5]. Apoptosis is defined as cell death activated by an internally controlled suicide program that bacteria are able to trigger apoptosis, including the secretion of compounds such as protein synthesis inhibitions, pore forming proteins, molecules responsible for the activation of the endogeneous death in the infected cell, and super antigens [6]. Regulation of apoptosis is essential for normal embryonic development and for homeostasis in adult tissue.

Zinc has a rather low toxicity and influences apoptosis by acting on several molecular regulators of programmed cell death which can inhibit apoptosis thereby either prolonging the survival of infected cells such that the production of progeny virus is maximized or facilitating the establishment of virus persistence. The influence of zinc on apoptosis is very complex that variables in this complex network are tissue and cell type, zinc concentration, expression of zinc tranporters and zinc-binding proteins, oxidative or nitrosative stress, and the improvement of molecular opposing functions. The other, released Zn^{2+} ions from zinc complexes and zinc oxide (ZnO) nanoparticles (ZnONPs) have significant roles for cancer cell that angiogenesis refers to the budding of new capillary branches from existing bloods vessels. Angiogenesis is involved not only with normal and developmental physiological processes, but also in the development of a number of pathological conditions, including rheumatoid arthritis, psoriasis, retinopathies and cancer [7]. Zinc-dependent antibacterial vaccine principle has been not completely understood, but novel research as targets for antibacterial vaccines and therapies has been proceeding [8,9]. Zinc ion killing occurs chiefly by bacteriolyses of bacterial cell walls due to activated peptidoglycan (PGN) autolysins such as amidases, endopeptidases, and carboxypeptidase against bacteria [10]. These PGN autolysins induced anti-bacterial vaccine activity may be enhanced by activation of zinc dependent PGN autolysins. PGN autolysins are bacterial peptidoglycan degrading enzymes that these muropeptides can be produced or modified by the activity of bacterial glycolytic and peptidolytic enzymes referred to as PGN hydrolases and autolysins which specific bacterial pathogens use PGN degradation to subvert host innate immunity [11].

Zinc homeostasis during acute phase response is the temporal transfer of serum zinc to the tissues, causing transient serum hypozincemia, which is rebalanced during resolution of the inflammatory response that intracellularly increased zinc can intoxicate engulfed pathogens and acts cytoprotective by promotion of neutralizing Reactive Oxygen Species (ROS) and nitrogen species (RNS) [12]. Bacteria have to avoid recognition by the host immune system in order to establish a successful infection which bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system [13].

In this review, anti-bacterial vaccine activities of Zn, ZnO nanoparticles and bacteriolyses by Zn^{2+} ions induced PGN autolysin activations are discussed against Gram-positive and Gram-negative bacteria. Thereby, the zinc-mediated molecular vaccine mechanisms may be clalified.

Anti-bacterial vaccine activities of Zn and ZnO-NPs against *Gram-positive bacteria*

Zinc is used as a structural or catalytic cofactor in the wider number of proteins that the zinc uptake regulator (Zur) is the most wide-spread, in which Zur proteins govern zinc homeostasis in a much more profound way than merely through the expression of uptake systems [14]. Adsoption of Zn^{2+} ions to the bacterial cell surface increases cell wall cohesion and favors the projection of elongated *S. aureus* surface protein (SasG) away from the cell surface, thereby enabling zinc-dependent homophilic bonds between opposing cells that zinc-dependent cell surface dynamics may represent a general mechanism for activating adhesion in biofilm-forming species [15]. Important considerations in designing a vaccine for the prevention of *S. aureus* disease have been outlined, accopanying with the complexity of Staphylococcal diseases

and having aided design of new vaccine candidates based on multiple important bacterial pathogenesis mechanisms [16]. The mechanisms underlying *S.aureus* extracellular vesicles (EVs) production and highlights on the usefulness of EVs as *S. aureus* vaccine platform have been described [17].

Antibody and vaccine development against *S.aureus* that produces cell envelope-associated proteins, secreted toxin, host cell lysis antibody function interference, are physiologically and pathologically considered That Staphylococcal Entoxin Serotype B (SEB) and superantigenicity of superantigens (Sags) are largely achieved by the activated Antigen Presenting Cells (APCs) and T cells, leading to a massive release of cytokines [18]. In order to generate effective bacterial whole-cell vaccines auxotrophic for D-glutamate, it has been clear that the D-glutamate is effective for community accquired *MRSA,* and the other, it is efficient for *P. aeruginosa* PA14 [19].

Fusion protein consisting of most probable immunoprotective B-cell epitope regions (MIBRs) are both plasmid Stabilization Protein (PSP) and Zinc Binding Lipoprotein (ZBL), PSP and ZBL respectively (APZs), in which the autolysin MIBRs show the highest probability for eliciting immunoprotection and pneumococcal conjugate vaccine against *Streptococcus pneumoniae* [20]. *Clostridium difficile* Residues are important in zinc binding and enzymatic activity that CD630 28300 (named Zmp1) destabilizes the fibronectin network produced by human fibroblast which a novel extracellular zinc metalloprotease may be important in key steps of clostridial pathogenesis [21]. Immunization of mice with the extracellular component of the zinc importer confers protection against system GAS,and a similar struggle for zinc may occur during streptococcal infections [22]. Mice were immunized with the antibodies raised against recombinant lipoproteins, showing significant reduction of colony counts in mice levers and demontrating the efficacy of these metal binding lipoproteins as promising vaccine candidates [23]. Zinc supplimentation promotes the induction of T cell immunity to control infection and ameliorate immunopathology against Gram-positive pneumonia in children [24]. Zinc is an essential nutrient for microbial growth, but can be toxic in excess. Zinc importer adcABC of the primary group A streptococcus (GAS) zinc uptake system is composed of a cell surface-exposed zinc-binding protein (adcA), an inner membrane permease(AdcB), and a cytosolic ATPase (AdcC) that provides the energy for zinc import by ATP hydrolysis [25]. Pnemococcal choline binding proteins (CBPs) include cell wall hydrolases and play a dual role for the development of novel antipneumococcal drugs, both as targets for inhibitors of binding to the cell wall and as active cell lytic agents [26]. Human peptidoglycan recognition proteins (PGLYRPs) are novel class of recognition and effector molecules with broad Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria [27].

Zinc oxide (ZnO) nanoparticles (ZnONPs) of ZnO nanoparticles-dependent anti-bacterial vaccine are attractive antibacterial properties with broad-spectrum antibiotics due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. Bactericidal and bacteriostatic activity of ZnO-NPs is associated with the generation of Reactive Oxygen Species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), and peroxide (O_2^{-2}) that ROS have been a mjor factor for several mechanisms including cell wall damage due to ZnO-localized interaction, enhanced membrane permea-bility, internalization of Nps due to loss pf proton motive force and uptake of toxic dissolved zinc ions [28]. Zinc oxide is an essential ingredient of many enzymes,sun screens,and ointments for pain and itch relief that reliesed zinc ions from zinc oxide penetrate the bacterial cell wall via diffusion that ZnONPs disintegrate the cell membrane and accumulate in the cytoplasm where theyinteract with bio-molecules causing cell apoptosis leading to cell death [29]. ZnO-NPs against *MRSA* are that exposure to ZnO-NPs resulted in over three-log reduction in colonies of *MRSA* with minimal increase in ROS or lipid peroxidation which ZnO-NPs caused significant up-regulation of pyrimidine biosynthesis and carbohydrate degradation [30]. The antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress.

Molecular PGN structure of *S. aureus* and the action sites of PGN autolysins

Bacterial peptidoglycan (PGN) structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and β-(1-4)-N-acetylmuramic acid (NAM) that are crosslinked by peptide stem chains attached to the NAM residues [31]. As shown in (Figure 1), the action sites of bacterial autolysins are comprised that for *Staphylococcus aureus* (*S.aureus)* PGN layer cell wall, there are N-acetylmuramidase-L-

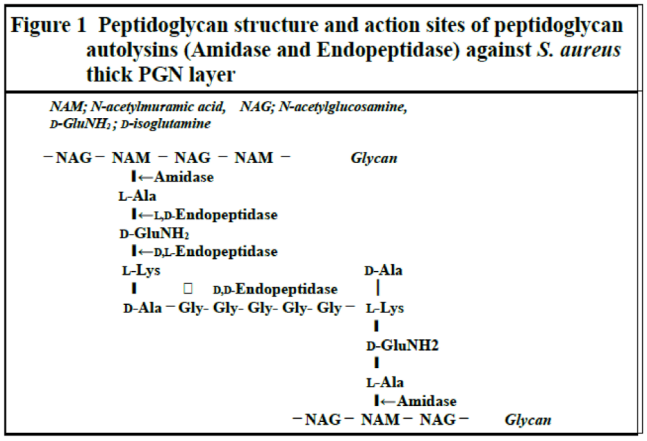

Figure 1 Peptidoglycan structure and action sites of peptidoglycan autolysins (Amidase and Endopeptidase) against *S. aureus* thick PGN layer

alanine amidase and DD-endopeptidase [32]. The bacterial cell walls are a strong flexible mesh work of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodeled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase, gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [33]. In these autolysins, zinc-dependent PGN autolysin of major amidases may be enhanced and induced the anti-bacterial vaccine activities.

Bacteriolyses of bacterial cell walls by Zn^{2+} ions-induced PGN autolysin activations promote the antibacterial vaccine activity against Gram-positive bacteria

S.aureus amidase AmiA is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against *MRSA* [34].

The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophyticus*) is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats [35].

Lytic amidase autolysin LytA which is released by bacterial lysis, associates with the cell wall via its zinc-binding motif that the amidase domain comprises a complex substrate-binding crevice and needs to interact with a large-motif epitope of PGN for catalysis [36]. Suicidal amidase autolysin LytA having both autolysis and capsule shedding depends on the cell wall hydrolytic activity of LytA that capsule shedding drastically increases invasion of epithelial cells and is the main pathway by which pneumococci reduce surface bound capsule during early acute lung infection of mice [37]. In the biofilms increase as zinc concentrations increase and biofilm formation effect as a negative regulator of LytA dependent autolysis, zinc availability contributes to the ability of pneumo-cocci to form aggregates and subsequently, biofilms [38]. The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc-β-(1,4)-MurNAc glycosidic bond of PGN building units that cell wall digestion products and solubilisation rates might indicate a tight control of LytB activity to prevent unrestrained breakdown of the cell wall [39]. The PGN-remodeling autolysins LytC, LytD, and LytF are expressed in the same subpopuration of cells and complete flagellar synthesis that LytC appears to be important for flagellar function, motility was restored to a LytC mutant by mutation oef either lon A, and LytC, LytD,and LytF autolysins to population heterogeneity in *B.subtilis* [40]. Atl is the major autolysin in *S aureus* that the bifunctional major autolysin play a key role in staphylococcal cell separation which processing of Atl yield catalytically active amidase and glucosamidase domains [41]. The biochemical and strucural staphylococcal Atl have successful cloaning, high level over-expression, and purification Atl proteins [42]. Major Atl autolysin also have an essential role in the early events of the fibronectin-binding proteins (FnBPs)-dependent *S.aureus* biofilm phenotype [43]. Furthemore, it is worth noting as a novel recombinant vaccine candidate comprising penicilline-binding protein2a (PBP2a) and r-autolysin that active vaccination with a mixture of r-PBP2a/r-autolysin and conjugate form vaccine reduced the mortality rate and protected mice against lethal *MRSA* [44]. For the contribution of autolysins of PGN hydrolases to bacterial killing, there are N-acetylglucosaminidase(AtlA), two N-acetyl-muraminases (AtlB and AtlC) [45]. AtlA is the major PGN hydrolases of *Enterro-coccus faecalis* involved in cell division and cellular autolysis and the zinc metalloprotease, gelatinase (GelE) of their interplay proposed to regulate AtlA function, which N-terminal cleavage was required for efficient AtlA-mediated cell division, and AtlA septum localization and subsequent cell separation can be modulated by a single GelE-mediated N-terminal cleaveage event [46]. Autolysin-mediated lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities.

(Table 1) represents anti-bacterial vaccine activities of Zn, ZnONPs and bacteriolysis by Zn2+ ions-induced PGN autolytic activation against Gram-positive bacteria PGN-layer cell wall.

Table 1: Antibacterial vaccine activities of Zn-, ZnONPs and Zn^{2+}ions-induced activated PGN autolysins against *Gram-positive*thick PGN envelope cell wall

Zn^{2+}Ions	*Gram-Positive*PGN Layer Cell Wall
Zn^{2+}	→ Zn^{2+}, O_2^-, H_2O_2, OH, NO, $ONOO^-$
	Zn, ZnONPs dependent antibacterial vaccine activities
	• MDR of Gram-positive strain as antibody and vaccine
	• Human PGLYRPs against both Gram-positive and Gram-negative bacteria
	• D-glutamate auxotrophy against *MRSA*
	• Extracellular zinc metalloprotease against *Clostridium difficile*
	• Zinc binding lipoprotein against *Enterococcal*infections
	• Zinc supplimentation for *pneumonia*in children
	• Zn^{2+}-dependent *S.aureus*surface protein (SasG) formation
	• Zinc importer AdcABC for streptococcal infections against *GAS*
	• *Pneumococcal CBPs*cell wall hydrolases
	• ZnO-NPs have a very high anti-bacterial activity and ROS generation gainst *MRSA* (ROS; H_2O_2, $OH^{\square}$, O_2^{-2})
	• ZnO-NPs caused up-regulation of pyrimidine biosynthesis and degradation against*MRSA*
	Antibacterial vaccine activities of bacteriolysis by Zn^{2+}ions induced activated PGN autolysins
	• *S. aureus*amidase AmiA
	• Recombinant amidase of the *Aas*
	• Lytic amidase LytA for *Streptococcus pneumoniae*
	• Pneumococcal autolysin LytALytC, D, F of PGN remodeling for *Bacillus subtilis*
	• Endopeptidase LytF for*bacillus subtilis*
	• AtlA autolysin for GelE against *E. faecalis*
	• AtlA,AtlB, AtlC autolysins against *enterococcus faecalis*
	• Fusion protein autolysin,*MIBRs* against *S. pneumoniae*
	• Carboxypeptidase B1 agsinst Anopheles stephensi and for*malaria*as transmission-blocking vaccines
	• Metallocarboxypeptidase M32 against*Trypanosoma brucei or cruzi*
	• PBP2a and autolysin mixture aginst*MRSA*

Anti-bacterial vaccine activities of Zn and ZnO-NPs against Gram-negative bacteria

Bacterial pathogens must produce high affinity zinc importers in order to grow and multiply in the infected host, such as the ZnuABC trasporter which is present in most Gram-negative bacteria, in which the disruption of ZnuABC transporter is usually associated with a remarkable loss of pathogenicity [47]. Neisserial outer-membrane transporter ZnuD is required for efficient systemic infection by the causative agent of bacterial *Neisseria meningitidis* [48]. ZnuD constitutes promising candidate for the development of a vaccine against meningoccal disease [49].

Antibody and vaccine activity against *E. coli* have been clarified that enterotoxigenic *E.coli* (ETEC) is the most common bacterial cause of children's diarrhea, in which antigen preparation induced antitoxin antibodies that neutralized both toxins that are associated with all cases of ETEC diarrhea, and polypeptide or subunit vaccines have the potential to effectively protect against ETEC diarrhea [50]. Oral vaccines which are intended for global use do not necessarily induce the same immune responses in all children worldwide that vaccine designed for oral administration will need to be adjusted to these potential problems in order to maximize benefits for all children [51]. Zinc has positive effect in children with complication of diarrhea that young children are immunized with oral inactivated whole cell cholera vaccine containing recombinant cholera toxin B subnit, in which the combination of zinc with cholera vaccine and Oral Rehydration Solutions (ORSs) has a positive impact on cholera and diarrhea [52]. Acute diarrhea remains a leading cause of childhood death despite the undenirable success of oral rehydration therapy (ORT) that Vaccination is the most effective method of preventing infectious diseases [53].There may be an influence of zinc on cholera vaccination and a suppresion of antibody formation against cholera toxin.

Zinc uptake A (ZnuA) is a high affinity acquisition of Zn^{2+} in *E. coli* was shown to occur via the ATP-binding cassette (ABC) permease and ZnuABC that the Znu permease comprises the solute-binding protein (SBP) ZnuA, and an ABC tranporter. The acquisition of zinc by *P. aeruginosa* PAO_1 reveals a hitherrto unrecognized complexity in zinc homeostasis that enables the bacterium to survive under zinc limitation that the mechanisms and pathways uitilyzed by *P. aeruginosa* to survive and promulgate in environments of varying Zn^{2+} abundance, with the findings widely applicable to other prokaryotic organisms [54]. Recombinant flagella and pili to targeting lipo-polysacharides and O-antigens have shown some promise in a preventing infection that outer membrane

protein including OprF and OprI are newer representative of vaccine candidates which many of the aforementined vaccine act on a single target, thus lacking a broad range of protection [55]. Recombinant AfeA expresses abundant epitopes on the bacterial surface and induces protective responses in the mouse pulmonary clearance model following aerosol challenge with *Moraxella catarrhalis,* in which AfeA is an excellent vaccine antigen to be included in a vaccine to prevent infections caused by *M.catarrhalis* [56]. Multivalent fusion DNA vaccine against *Brucella abortus* has been constructed that the expression of BAB antigens, encoded in B.abortus BAB1 0279 Open Reading Frame (ORF) genomic island 3 (GI-3) and conjugated to SOD protein can polarize mice immunity to a Th1-type phenotype, conferring low levels of protection in animal model [57].

ZnO-NPs disrupt the cell membrane and oxidative stress against *Campylobacter* [58]. ZnO-NP could be developed as alternative therapeutics against *A. baumannii* of a Gram-negative bacterium [59].

Molecular structures of *E. coli* and action sites of degrading enzyme of outer membrane lipoprotein at C- and N-terminals and PGN autolysins

As shown in Figure 2 for *Escherichia coli* (*E. coli)* cell wall, there are endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals, and amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space [32]. The bacterial cell walls are a strong flexible mesh work of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodeled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase, gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [33].

Zn^{2+} ions-induced degrading enzyme of outer membrane lipoprotein and activated PGN autolysins promote anti-bacterial vaccine activity against *Gram-negative* bacteria

Amidase gene (AmiB) catalyzes the degradation of PGN in bacteria that the amiB gene was composed of 1,722 nucleotides and 573 amino acid which is involved in the separation of daughter cells after cell devision and inactivation of the amiB gene, resulting in marked increases of sensitivity to oxidative stress and organic acids [60]. Amidase activity of amiC controls cell separation and PGN fragments release [61].

Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth that zinc avaliability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria [62]. Zinc-regulated peptidase maintains cell wall integrity during immune-mediated nutrient sequestration against *Acinetobacter baumannii* [63].

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxypeptidase B1 of and its evaluation have been high molecular characterization for tranmission-blocking vaccines (TBVs) against Malaria eradication [64]. Metallocarboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi* [65]. Thus, zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial vaccines (Table 2).

Conclusions

Anti-bacterial vaccine activities of Zn, ZnONPs and bacteriolyses by Zn^{2+} ions-induced activated PGN autolysins are discussed against Gram-positive and Gram-negative bacteria, and thereby the zinc-mediated molecular vaccine mechanisms have been clarified.

(1) For Gram-positive bacteria, antibody and vaccine development against *S.aureus* that produces cell envelope-associated proteins, secreted toxin, host cell lysis antibody function interference, are physiologically and pathologically considered that SEB and superantigenicity of superantigens (Sags) are largely achieved by the activated APCs and T cells, leading to a massive release of cytokines. In order to generate effective bacterial whole-cell vaccines auxotrophic for D-glutamate, it has been clear that the D-glutamate is effective for community accquired *MRSA,* and the other, it is efficient for *P. aeruginosa* PA14.

Fusion protein consisting of MIBRs are both PSP and ZBL, PSP and ZBL respectively (APZs), in which the autolysin MIBRs show the highest probability for eliciting immunoprotection and pneumococcal conjugate vaccine

Table 2: Antibacterial vaccine activity of Zn, ZnONPs, and Zn^{2+}-induced autolysin activations for *Gram-negsative* cell wall with outer membrane lipoprotein and thin PGN layer in periplasmic space

Zn^{2+}Ions	***Gram-negative*cell wall**	
Zn^{2+}	Outer Membrane Lipoprotein at C- and N-terminals → Zn^{2+}, $O_2^{\square}$, H_2O_2 **Zn and ZnONPs dependent antibacterial vaccine** • Outer membrane receptor against N.menigitidis • ETEC subunit vaccine • Oral vaccine by ORT • ZnuB against *P. aeruginosa.* • *Preventive vaccine by recombinan flagella against P. aeruginosa* **Bacteriolytic vaccine by Zn^{2+}ions - induced PGN autolysin activation** • Amidase gene amiB/LysM • Endopeptidase regulation of ShyA and *ShyB*	Periplasmic Space Thin PGN Layer →Zn^{2+}, $O_2^{\square}$, H_2O_2, $OH^{\square}$, $^{\square}OH$ **Zn and ZnONPs dependent antibacterial vaccine** • PGRPs or PGLYRPs • D-glutamate auxotrophy against *P. aeruginosa*PA14 • ORT in infectious *diarrhoea* • *ZnuA against P. aeruginosa* • Recombinant flagella and pili against *P. aeruginosa* **Bacteriolytic vaccine by Zn^{2+}ions-induced *PGN autolysin activation*** • AmiC inPGN fragment release • Carboxypeptidase by transmission - *blocking vaccines*
	• ZnO-NPs disrupt the cell membrane and oxidative stress againstCampylobacter • Combination of zinc and cholera vaccine and ORS • AfeA excellent vaccine antigen preventing infection of M.catarrhalis • Fusion DNA vaccine againstBrucella abortus	

against *Streptococcus pneumoniae*. *Clostridium difficile* Residues are important in zinc binding and enzymatic activity that CD630 28300 (named Zmp1) destabilizes the fibronectin network produced by human fibroblast which a novel extracellular zinc metalloprotease may be important in key steps of clostridial pathogenesis. Immunization of mice with the extracellular component of the zinc importer confers protection against system GAS,and a similar struggle for zinc may occur during streptococcal infections. Zinc supplimentation promotes the induction of T cell immunity to control infection and ameliorate immunopathology against Gram-positive pneumonia in children. Zinc is an essential nutrient for microbial growth. Zinc importer adcABC of the primary GAS zinc uptake system is composed of a cell surface-exposed zinc-binding protein (adcA), an inner membrane permease(AdcB), and a cytosolic ATPase (AdcC) that provides the energy for zinc import by ATP hydrolysis. Pnemococcal choline binding proteins (CBPs) include cell wall hydrolases and play a dual role for the development of novel antipneumococcal drugs, both as targets for inhibitors of binding to the cell wall and as active cell lytic agents. Adsoption of Zn^{2+} ions to the bacterial cell surface increases cell wall cohesion and favors the projection of elongated SasG away from the cell surface, thereby enabling zinc-dependent homophilic bonds between opposing cells that zinc-dependent cell

surface dynamics may represent a general mechanism for activating adhesion. Important considerations in designing a vaccine for the prevention of *S. aureus* disease have been accopanied with the complexity of Staphylococcal diseases and having aided design of new vaccine candidates based on multiple important bacterial pathogenesis mechanisms, in which the mechanisms underlying *S.aureus* EVs production and highlights on the usefulness of EVs as *S. aureus* vaccine platform.

ZnO nanoparticles-dependent anti-bacterial vaccine are attractive antibacterial properties with broad-spectrum antibiotics due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. Bactericidal and bacteriostatic activity of ZnO-NPs is associated with the generation of ROS including H_2O_2, $OH^{\square}$, and O_2^{-2}. Zinc oxide is an essential ingredient of many enzymes, sun screens, and ointments for pain and itch relief that reliesed zinc ions from zinc oxide penetrate the bacterial cell wall via diffusion that ZnONPs disintegrate the cell membrane and accumulate in the cytoplasm where they interact with bio-molecules causing cell apoptosis leading to cell death. ZnO-NPs against *MRSA* are that exposure to ZnO-NPs resulted in over three-log reduction in colonies of *MRSA* with minimal increase in ROS or lipid peroxidation which ZnO-NPs caused significant up-regulation of pyrimidine biosynthesis and carbohydrate degradation. The antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress.

Bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by

bacterial innate immune system. Autolysin mediated bacteriolysis- and zinc dependent lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities, where PGN autolysins interact with biomolecules causing cell apoptosis leading to cell death. The autolytic activity of the recombinant amidase of the Aas is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats. AmiB catalyzes the degradation of PGN in bacteria. Amidase activity of amiC controls cell ceparation and PGN fragments release. Lytic amidase autolysin LytA which is released by bacterial lysis, associates with the cell wall via its zinc-binding motif that the amidase domain comprises a complex substrate-binding crevice and needs to interact with a large-motif epitope of PGN for catalysis.The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc-β-(1,4)-MurNAc glycosidic bond of PGN building units. The PGN-remodeling autolysins LytC, LytD, and LytF are expressed in the same subpopuration of cells and complete flagellar synthesis. Accordingly, Zn^{2+} ions under the homeostasis region could be apprecicable for anti-bacterial vaccine development. *S.aureus* amidase AmiA of PGN autolysin is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated molecule, developing new therapeutics against *MRSA.*

(2) For Gram-negative bacteria, ZnuA is a high affinity acquisition of Zn^{2+} in *E. coli* was shown to occur via the ABC permease that the Znu permease comprises the SBP ZnuA, and an ABC tranporter. The acquisition of zinc by *P. aeruginosa* PAO_1 reveals a hitherrto unrecognized complexity in zinc homeostasis that enables the bacterium to survive under zinc limitation that the mechanisms and pathways uitilyzed by *P. aeruginosa* to survive and promulgate in environments of varying Zn^{2+} abundance. Recombinant *flagella* and pili to targeting lipo-polysacharides and O-antigens have shown some promise in a preventing infection that outer membrane protein including OprF and OprI are newer representative of vaccine candidates which many of the aforementined vaccine act on a single targe. Recombinant AfeA expresses abundant epitopes on the bacterial surface and induces protective responses in the mouse pulmonary clearance model following aerosol challenge with *Moraxella catarrhalis.*

ZnO-NPs disrupt the cell membrane and oxidative stress against *Campylobacter*. ZnO-NP could be developed as alternative therapeutics against *A. baumannii* of a Gram-negative bacterium. The antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress.

Amidase gene (AmiB) catalyzes the degradation of PGN in Gram-negative bacteria that the amiB is involved in the separation of daughter cells after cell devision and inactivation of the amiB gene, resulting in a marked increases of sensitivity to oxidative stress and organic acids. AmiC controls cell separation and PGN fragments release.

Zinc-dependent endopeptidases are predicted to hydrolyze PGN to facilitate cell growth that zinc avaliability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria. Zinc-regulated peptidase maintains cell wall integrity during immune-mediated nutrient sequestration against *Acinetobacter baumannii*.

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxypeptidase B1 of and its evaluation have been high molecular characterization for TBVs against Malaria eradication. MCPs of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi*. Thus, zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial vaccines.

(3) For the molecular mechanisms, adsoption of Zn^{2+} ions to the bacterial cell surface increases cell wall cohesion and favors the projection of elongated SasG away from the cell surface, thereby enabling zinc-dependent homophilic bonds between opposing cells that zinc-dependent cell surface dynamics may represent a general mechanism for activating adhesion. Released zinc ions from zinc oxide penetrate the bacterial cell wall via diffusion that ZnO-NPs disintegrate the cell membrane and accumulate in the cytoplasm.

Important considerations in designing a vaccine for the prevention of infective disease have been accompanied with the complexity of such as Staphylococcal diseases and having aided design of new vaccine candidates based on multiple important bacterial pathogenesis mechanisms, in which the mechanisms underlying EVs production and

highlights on the usefulness of EVs as bacterial vaccine platform. Antibody and vaccine development that produces cell envelope-associated proteins, secreted toxin, host cell lysis antibody function interference,are physiologically and pathologically considered that SEB and superantigenicity of Sags are largely achieved by the activated APCs and T cells, leading to a massive release of cytokines. Human PGLYRPs are novel class of recognition and effector molecules with broad Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria. Thus, Zn-,ZnONPs-dependent anti-bacterial cell wall destruction and autolysin-mediated lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities.

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