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Zinc-Induced Immune Anti-Bacterial Vaccine Activity in Bacterial Cell Walls Against Gram-Positive and Gram-Negative Bacteria

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Abstract: *Zinc-induced immune anti-bacterial vaccine activities of bacteriolyses of the bacterial cell walls are discussed on Zn 2+ ions-induced activated PGN autolysins and ZnO-nanoparticles, and zinc-induced anti-bacterial vaccine mechanism can be clarified.Zinc homeo- stasis is the temporal transfer of serum zinc to the tissues, causing transient serum hypozincemia, which is rebalanced during resolution of the inflammatory response. Bacteria have to avoid recognition by the host immune system in order to establish a succesful infection which bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system. The bacterial cell walls are remodeled by PGN synthesis and PGN autolysin. S.aureus amidase AmiA is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of Staphylococcus saprophyticus) is inhibited. AmiB catalyzes the degradation of PGN in bacteria, resulting in marked increases of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell ceparation and PGN fragments release. Autolysin-mediated lysis-induced bacterial cell death can contribute to the bactericidal 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 endo- peptidase LytF are expressed in the same subpopuration of cells and complete flagellar synthesis. Major Atl autolysin also has an essential role in the early events of the fibronectin-binding proteins (FnBPs)-dependent S.aureus biofilm phenotype. Human peptidiglycan recognition proteins (PGLYRPs) are novel class of recognition and effector molecules with broad Zn2+-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria. Enterotoxigenic E.coli (ETEC) is the most common bacerial cause of children's diarrea that are associated with all cases of ETEC diarrhea, and polypeptide or subunit vaccines have the potential to effectively protect against ETEC diarrhea. 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. Amidase amiC controls cell ceparation and PGN fragments release against Neisseria genorrhoeae. Zinc uptake A (ZnuA) is a high affinity acquisition of Zn2+ in E. coli was demonstrated and shown to occur via the ATP-binding cassette (ABC) permease, ZnuABC that the Znu permease comprises the solute-binding protein (SBP) ZnuA. The acquisition of zinc by P. aeruginosa PAO1 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 Zn2+ abundance. Recombinant flagella and pili to targeting lipo-polysacharides and O-antigens have shown some promise in an preventing infection that outer membrane protein including OprF and OprI are newer representative of vaccine candidates. Recon- binant AfeA expresses abundant epitopes on the bacterial surface and induces protective responses in the mouse pulmonary clearance model following aerosol challenge with Moraxella catarrhalis. Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth and carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides and its evaluation has been high molecular characterization for transmission-blocking vaccines (TBVs). Bacteriolytic activity of ZnO-NPs is associated with the generation of reactive oxygen species (ROS) including hydrogen peroxide (H2O2), hydroxyl radicals (OH ⁻), and peroxide (O -2) that ROS have been cell wall damage due to ZnO-localized interaction. Reliesed zinc ions from zinc oxide penetrate the bacterial cell wall via diffusion that ZnO-NPs disintegrate the cell membrane and accumulate in the cytoplasm where they interact with biomolecules causing cell apoptosis leading to cell death. ZnO-NPs caused significant up-regulation of biosynthesis and degradation. Hence, the anti-bacterial mechanisms are due to the bacteriolyses by Zn2+ ions-induced activated PGN autolysin of amidases and the ZnO-NPs's disruption of cell membrane and oxidative stress. Accordingly, oral rehydration therapy (ORT) vaccination that Zn2+ ions status under the homeostasis region could be appreciable for anti-bacterial vaccine development, may be the most effective method of preventing infectious diseases.*

Keywords: *Zinc immunity, Zn^{2+}-induced anti-bacterial vaccine, PGN hydrolase and autolysin, Amidase/Endopeptidase/ Carboxypeptidase autolysins, ZnO-NPs.*

Abbreviations: *Aas=autolysin/adhesin of Staphylococcus saprophytic us, 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, E. coli=Escherichia coli, E. faecalis=Enterococcus faecalis, E. faecium=Enterococcus faecium,* ETEC= Enterotoxigenic *E.coli, Eps=Zinc dependent endopeptidases, FnBPs=*fibronectin-binding proteins, *Gas=group A streptococcus, GelE=zinc metalloprotease, gelatinase, M.catarrhalis= Moraxella catarrhalis, MCPs=Metallo carboxy peptidases, MIBRs=most probable immunoprotective B-cell epitope regions, MRB=multidrug bacteria, MRSA=methicillin-resistant Staphylococcus aureus, 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, ZnO-NPs=Zinc oxide (ZnO) nanoparticles, ZBL=zinc binding lipoprotein, Znu A=Zinc uptake A.*

1. INTRODUCTION

Zinc plays an important role in human immunity. 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 [1]. Zinc deficiency causes severe impairment of immune function, comprising the adaptive as well as the innate immune system, adequate zinc homeostasis is essential for a well-functioning immune system, and high zinc excess provokes an impairment of the immune system comparable to zinc deficiency [2]. Zinc deficiency in the host is linked to increased susceptibility to bacterial infection, zinc homeostasis must be essential to be maintained for bacteria, and zinc excess is highly toxic toward microorganism in an anti-bacterial role for zinc in innate immune defense against infected diseases [3].

The role of zinc in cell death has apoptosis that 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 [4]. The influence of zinc on apoptosis is tissue/cell type, zinc concent-ration, and expression of zinc transporters and zinc-binding proteins [1]. 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 transporters and zinc-binding proteins, oxidative or nitrosamine stress, and the improvement of molecular opposing functions. Zinc-dependent antibacterial vaccine principle has been not completely understood, but novel research as targets for antibacterial vaccines and therapies has been proceeding [5, 6].

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 [7]. 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.

Bacterial killing by zinc ions occurs chiefly by bacteriolyses of bacterial cell walls due to zinc ions-induced activated peptido-glycan (PGN) autolysins such as amidases, endopeptidases, and carboxypeptidase against bacteria [8]. PGN autolysins are bacterial PGN 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 [9]. These zinc ions-induced PGN autolysins may be activated and led to be applied towards anti-bacterial vaccine activities. In this review, firstly, zinc immunity in fection is outlined on the immune response in the regulation of the innate immune system. Secondly. Zn^{2+} ions-induced immune anti-bacterial vaccine activities for bacteriolysis by zinc-induced activated PGN autoly-sins are discussed against Gram-positive PGN layer cell wall and Gram-negative cell wall consisting of outer membrane lipoprotein and PGN thin layer

in periplasm space. Lastly, ZnO nanoparticles-dependent anti-bacterial vaccine is argued and the zinc-dependent molecular vaccine mechanisms could be calcified.

2. Zinc Immunity in Infection

The innate immune system represents the defense first line against a pathogen before the adaptive system can develop the appropriate response. Many organs are affected by zinc deficiency, especially the immune system that is markedly susceptible to changes of zinc levels which the immune response involves in the regulation of the innate and adaptive immunity, and this zinc homeostasis is critical for sustaining proper immune function [10].

Thus, inflammation is a natural process required to protect the host from tissue damage and infections, which leads to the resolution of the inflammatory response and the restoration of homeostasis. Despite zinc deficiency can be treated by proper zinc intake, suboptimal zinc status cannot simply diagnosed by reason of the lack of clinical sighns and reliable biochemical indicators of zinc status. High zinc concentration is that zinc binding to proteins can activate or inactivate thir activity, or change characteristics important for substrate binding, whiles, zinc homeostasis is primarily controlled via the expression and action of 14 zinc transporters that decreasing cytoplasm zinc can describe export via ZnTs, but also the transport of zinc into one of those organelles [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 intracellular increased zinc can intoxicate engulfed pathogens and acts cytoprotective by promotion of neutralizing reactive oxygen species (ROS) and nitrogen species (RNS) [11]. 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 peptidoglycan (PGN) to prevent detection by bacterial innate immune system [12].

3. Zn2+ Ions-Induced PGN Autolytic Activation Enhances the Anti-Bacterial Vaccine Activity

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 cross linked by peptide stem chains attached to the NAM residues [13]. The action sites of bacterial autolysins are comprised that for *S.aureus* PGN layer cell wall, there are N-acetylmuramidase-L-alanine amidase and DD-endopeptidase, and the other, for *E. coli* cell wall, there are end peptidase of degrading enzyme at lipoprotein of C- and N-terminals, and also amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space [14]. The bacterial cell walls are a strong flexible meshwork 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. In these autolysins, the PGN amidases that are activated by Zn^{2+} ions-induced PGN autolysin may promote the anti-bacterial vaccine activities. 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, glycosidase, and peptidase display a modular architecture combin-ing multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [15].

4. Zn2+ Ions-Induced Anti-Bacterial Vaccine Activity by Bacteriolyses of Gram-Positive Thick PGN Layer Cell Wall

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* [16].

The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophytic us*) is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats [17]. 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 a marked increase of sensitivity to oxidative stress andorganic acids [18]. Amidase activity of amiC controls cell ceparation and PGN fragments release [19].

Autolysin-mediated lysis-induced bacterial cell death can contribute to the bactericidal vaccine

activities. 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 [20]. 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 [21]. 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 pneumococci to form aggregates and subsequently, biofilms [22]. 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 [23]. 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 ***Endopeptidase LytF*** autolysins to population heterogeneity in *B. subtilis* [24].

Atl is the major autolysin in *S aureus* that the bifunctional major autolysin plays a key role in staphylococcal cell separation which processing of Atl yield catalytically active amidase and glucosamidase domains [25]. The biochemical and strucural staphylococcal Atl have successful cloaning, high level over-expression, and purification Atl proteins [26]. Major Atl autolysin also has an essential role in the early events of the fibronectin-binding proteins (FnBPs)-dependent *S.aureus* biofilm phenotype [27].

For the contribution of autolysins of PGN hydrolases to bacterial killing, there is N-acetylglucosaminidase (AtlA), two N-acetyl-muraminases (AtlB and AtlC) [28]. AtlA is the major PGN hydrolases of *Enterrococcus 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 subsequnt cell separation can be modulated by a single GelE-mediated N-terminal cleaveage event [29].

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 (APCs) and T cells, leading to a massive release of cytokines [30]. 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 [31]. 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 [32].

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 clostridal pathogenesis [33]. Mice were immunized with the antibodies raised against reconbinant lipoproteins, showing significant reduction of colony counts in mice levers and demonstrating the efficacy of these metal binding lipoproteins as promising vaccine candidates [34]. Zinc supplimentation promotes the induction of T cell immunity to control infection and amelio-rate immunopatho-logy against Gram-positive pneumonia in children [35]. 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 [36]. 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 [37]. 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 [37]. Extracellular vesicles (EVs) are immunogenic in mice, elicit cytolysin-neutralizing antibodies, and protect the animals in a lethal sepsis model that mechanisms underlie *S.aureus* EV production and highlights the usefulness of Evs as a *S. aureus* vaccine platform [38]. 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 [39]. 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 pneumo-coccal conjugate vaccine against *Streptococcus pneumoniae*[40].

5. ZN2+ IONS-INDUCED ANTIBACTERIAL VACCINE ACTIVITY BY BACTERIOLYSES OF GRAM-NEGATIVE BACTERIA CELL WALL CONSIST-ING OF OUTER MEMBRANE LIPOPROTEIN AND PGN THIN LAYER IN PERIPLASM SPACE

Antibody and vccine activity against *E. coli* have been clarified that enterotoxigenic *E.coli* (ETEC) is the most common bacerial cause of children's diarrea, 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 [41]. Amidase amiC controls cell ceparation and PGN fragments release against *Neisseria genorrhoeae* [19]. 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 [42]. Zinc has positive effect in children with compli-cation of diarrhea that young children are immunized with oral inactiva-ted 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 [43]. 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 [44]. 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 promul-gate in environments of varying Zn^{2+} abundance, with the findings widely applicable to other prokaryotic organisms [45]. 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 aforementioned vaccine act on a single target, thus lacking a broad range of protection [46]. 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* [47]. 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 [48]. 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 [49].

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C

terminus of proteins or peptides that the carboxypeptidase B1 (*Anopheles stephensi*) and its evaluation have been high molecular characterization for tranmission-blocking vaccines (TBVs) against Malaria eradication [50]. Metallocarboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi* [51].

Thus, zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial vaccines. 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* [52].

6. ZNO NANOPARTICLES-DEPENDENT ANTI-BACTERIAL VACCINE

Zinc oxide (ZnO) nanoparticles (ZnO-NPs) are attractive anti-bacterial properties with broad-spectrum antibiotics due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. Bactericidal and bacterio-static activity of ZnO-NPs is associated with the generation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), 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 permeability, internalization of Nps due to loss pf proton motive force and uptake of toxic dissolved zinc ions [53]. 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 ZnO-NPs disintegrate the cell membrane and accumulate in the cytoplasm where theyinteract with biomolecules causing cell apoptosis leading to cell death [54]. 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 per-oxidation which ZnO-NPs caused significant up-regulation of pyrimidine biosynthesis and carbohydrate degradation [55]. The antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress.

Table1: *represents summarily anti-bacterial vaccine activities of bacteriolyses by Zn^{2+} ions-induced PGN activated autolysins and ZnO nanoparticles against Gram-positive bacteria PGN envelope cell wall and Gram-negative outer membrane lipoprotein and thin PGN layer of periplasmic space in cell wall.*

Zn^{2+} Ions	***Gram-Positive* PGN Layer Cell Wall**	
Zn^{2+}	Zn^{2+} ions induced PGN autolysins - Zn2+, O2 , H2O2, ·OH, ·NO, ONOO ⁻ Zn^{2+} ions-induced activated PGN autolysins ·*S.aureus* amidase AmiA ·Recombinant amidase of the *Aas* ·Lytic amidase LytA for *Streptococcus pneumoniae* ·*Pneumococcal autolysin* LytA LytC, 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* ·Metallocarboxypeptidase M32 against *Trypanosoma brucei or cruzi* ·PBP2a and autolysin mixture aginst *MRSA* ·*ROS and RNS generations(Zinc homeostasis)* ·ZnO-NPs have a very high anti-bacterial activity and ROS generation gainst *MRSA* (ROS; H_2O_2, OH^-, O_2)-2 ·ZnO-NPs caused up-regulation of pyrimidine biosynthesis and degradation agasinst *MRSA*	
Zn^{2+} ions	***Gram-Negative* Cell Wall**	
	Outer Membrane Lipoprotein at C- and N-terminals	Periplasmic Space Thin PGN Layer
	→ Zn^{2+}, O_2 , H_2O_2 ⁻	→ Zn^{2+}, O_2 , H_2O_2, OH , ·OH⁻ ⁻

Zn^{2+}	·Amidase gene amiB/LysM ·Endopeptidase regulation of ShyA and ShyB ·Outer membrane receptor against N.menigitidis ·ETEC subunit vaccine ·ZnuB against *P. aeruginosa*. ·Preventive vaccine by recombinant flagella *against P. aeruginosa* ·*ROS and RNS*	·AmiC in PGN fragment release ·Carboxypeptidase by transmission-blocking vaccines ·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*、*ROS and RNS*
	·ZnO-NPs disrupt the cell membrane and oxidative stress against *Campylobacter*	

7. CONCLUSIONS

Bacteria have to avoid recognition by the host immune system in order to establish a succesful infection which bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system. Zinc homeostasis during acute phase response is the temporal transfer of serum zinc to the tissues, causing transient serum hypozin-cemia, which is rebalanced during resolution of the inflammatory response. The bacterial cell walls are remodeled by PGN synthesis and PGN autolysin. *S.aureus* amidase AmiA is functional on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated water molecule, developing new therapeutics against *MRSA*. 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. Amidase gene (AmiB) catalyzes the degradation of PGN in bacteria. Amidase activity of amiC controls cell ceparation and PGN fragments release.

Autolysin-mediated lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities. Lytic amidase autolysin LytA which is released by bacterial lysis, 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. The PGN-remodeling autolysins LytC, LytD, and endopeptidase LytF are expressed in the same subpopuration of cells and complete flagellar synthesis. Major Atl autolysin also has an essential role in the early events of the FnBPs-dependent *S.aureus* biofilm phenotype.

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. Amidase amiC controls cell ceparation and PGN fragments release against *Neisseria genorrhoeae.* 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 in biofilm-forming species. Zinc is an essential nutrient for microbial growth, but can be toxic in excess. 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.

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 ORSs has a positive impact on cholera and diarrhea. Acute diarrhea remains a leading cause of childhood death despite the undenirable success of ORT that vaccination is the most effective method of preventing infectious diseases.There may be an influence of zinc on *cholera* vaccination and a suppresion of antibody formation against *cholera* toxin.

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, with the findings widely applicable

to other prokaryotic organisms. Reconbinant 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*.

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.

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxypeptidase B1 (Anopheles stephensi) and its evaluation have been high molecular characterization for tranmission-blocking vaccines (TBVs) against Malaria eradication.

ZnO-NPs 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^-), 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 permeability, internalization of Nps due to loss pf proton motive force and uptake of toxic dissolved zinc ions. ZnO-NPs disintegrate the cell membrane and accumulate in the cytoplasm where they interact with bimolecular causing cell apoptosis leading to cell death. ZnO-NPs caused significant up-regulation of pyramiding biosynthesis and carbohydrate degradation.

Thus, as the bacterial cell walls are remodeled by PGN synthesis and PGN autolysin, Zn^{2+}-induced activated PGN autolytic amidase within these autolysins may enhance and promote the anti-bacterial vaccine activities. The other, anti-bacterial vaccine mechanism of ZnO-NPs is likely due to disruption of the cell membrane and occurrence of the oxidative stress.

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