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Zinc Immunology and Zn^{2+} Ions Binding Anti-Bacterial Vaccine Activity for Bacterial Cell Walls against Gram-Positive and Gram-Negative Bacteria

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Abstract

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. 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 shed light 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 (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 ceparation and PGN fragments release. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial vaccine activities.

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 LytF are expressed in the same subpopuration of cells and complete flagellar synthesis. Major Atl autolysin also have 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 Zn^{2+}-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria. It has been clear that the D-glutamate is effective for community accquired MRSA, and the other, it is efficient for P. aeruginosa PA14.

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. 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.

Enterotoxigenic E.coli (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. Oral vaccines which are intended for global use do not necessarily induce the same immune responses in all children worldwide. 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. 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.

Zinc uptake A (ZnuA) is a high affinity acquisition of Zn^{2+} 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, 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. 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 which many of the aforementined vaccine act on a single target, thus lacking a broad range of protection. 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.

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.

Zinc oxide (ZnO) nanoparticles (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 reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and peroxide (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. 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. The antibacterial mechanism of ZnO-NPs is likely due to disruption of the cell membrane and oxidative stress such as Campylobacter.

Accordingly, Zn^{2+} ions under the homeostasis region could be apprecicable for anti-bacterial vaccine development.

Keywords: *Zinc homeostasis, Zn^{2+}-binding vaccine, PGN hydrolase and autolysin, Amidase, ZnO-NPs, ROS.*

Abbreviations

Aas=autolysin/adhesi n of *Staphylococcus sapro phyticus*, **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 fae calis,* ***E. faecium=****Enterococcus faecium,* ***ETEC=****Enterotoxigenic E.coli*, **Eps=**Zinc dependent endope ptidases, **FnBPs=**fibronectin-binding proteins, **Gas=**group A streptococcus, **GelE=**zinc metall oprotease, 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 rehy- dration therapy, **P. aeruginosa=**Pseudomonas aer- uginosa, **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=** transmission-blocking vaccines, **VRE=**vancomycin-resistant Enterococcus faecium, **ZnO-NPs=**Zinc oxide (ZnO) nanoparticles, **ZBL=**zinc binding lipoprotein, **ZnuA=**Zinc uptake A.

Introduction

Zinc plays an important role in human immunity that 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 [1]. Zinc deficiency causes severe impairment of immune function, conprising 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 is the second most abundant trace metal with human body 2-3 g, 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 [3]. 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 [3]. Host zinc homeostasis changes in response to bacterial infections, including production of metal sequestering proteins and bombardment of bacteria with toxic level of zinc at host-pathogen interface [4]. 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 [5]. 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 there by 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 transporters and zinc-binding proteins,oxidative or nitrosative stress, and the improvement of molecular opposing functions. Zinc-dependent antibacterial vaccine principle has been not completely

understood, but novel research as targets for anti bacterial vaccines and therapies has been proceeding [6,7].

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 [8]. These PGN autolysins induced anti-bacterial vaccine activity may be enhanced by activation of zinc dependent PGN autolysins. 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].

In this review, Zn^{2+} ions binding anti-bacterial vaccine activity for PGN autolysins induced vaccine, zinc-dependent bacterial vaccine, and ZnO nanoparticle induced anti-bacterial vaccine are dicussed, and then, the zinc dependent molecular vaccine mechanisms are clalified.

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 cytoplasmic 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 intracellularly 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 succesful infection which bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system [12].

Zinc-Binding PGN Autolysin Enhanced 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-acetylmuramicacid (NAM) that are crosslinked 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 endopeptidase 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, zinc-dependent PGN autolysin of amidases may be enhanced and induced 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, gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [15].

S.aureus amidase AmiA shed light 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 saprophyticus*) 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 increases of sensitivity to oxidative stress and organic 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 LytF autolysins to population heterogeneity in *B. subtilis* [24].

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 [25]. The biochemical and strucural staphylococcal Atl have successful cloaning, high level over-expression, and purification Atl proteins [26]. Major Atl autolysin also have 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 are 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].

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 [30]. 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* [31].

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or peptides that the carboxy-peptidase B1 of and its evaluation have been high molecular characterization for transmission-blocking vaccines (TBVs) against Malaria eradication [32]. Metallocarboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei or cruzi* [33]. 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 [34].

Zn^{2+} Ions-Dependent Bacterial Cell Walls Induced Anti-Bacterial Vaccine Activity

Zn^{2+} Ions Binding Vaccine Activity for Thick PGN Cell Wall Against Gram-Positive Bacteria

Antibody and vaccine development against *S.aureus* that produces cell envelop-associated proteins, secreted toxin, host cell lysis antibody function interference, are physiologically and pathologically considered that staphylococcal entoxin serotype B (SEB) and super antigenicity of superantigens (Sags) are largely achieved by the activated (APCs) and T cells, leading to a massive release of cytokines [35]. 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 [36]. 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 [37].

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 [38]. Mice were immunized with the antibodies raised against reconbinant lipoproteins, showing significant reduction of colony counts in mice levers and demontrating the efficacy of these metal binding lipoproteins as promising vaccine candidates [39]. Zinc supplimentation promotes the induction of T cell immunity to control infection and ameliorate immunopatho-logy against Gram-positive pneumonia in children [40]. Adsorption 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 [41]. 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 [42]. 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 [42]. Extracellular vesicles(EVs) are immuno-genic 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 [43]. 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 [44].

Table 1 represents vaccine activities of Zn^{2+} ions for thick PGN layer cell wall against Gram-positive bacteria.

Table 1. *Zn^{2+} ions binding vaccine activity for Gram-positive PGN layer cell wall*

Zn^{2+}Ions	Gram-Positive Bacterial Thick PGN Cell Wall
Zn^{2+}ions	**Zinc-binding PGN autolysins, Zn2+-dependent bactericide** • **Zinc Ions-Dependent Autolysins** *S.aureusamidase AmiA* *Recobinant 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* *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* • 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 *pneumoniain* children • *Zn^{2+}-dependent S.aureussurface protein (SasG) formation* • *Zinc importer AdcABC for streptococcal infections against GAS* • *Pneumococcal CBP*scell wall hydrolases • ZnO-NPs have a very high anti-bacterial activity and ROS generation gainst MRSA (ROS; H2O2, OH^-, $O2^{-2}$) • ZnO-NPs caused up-regulation of pyrimidine biosynthesis and degradation agasinst *MRSA*

Zn^{2+} Ions Binding Vaccine Activity for Gram-Negative Bacterial Cell Wall Having 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 [45]. Oral vaccines which are

intended for global use do not necessarily induce the same immune responses in all children world wide that vaccine designed for oral administration will need to be adjusted to these potential problems in order to maximize benefits for all children [46]. Zinc has positive effect in children with complication of diarrhea that young children are immunized with oral inactiva-ted whole cell cholera vaccine containing recombinant cholera toxin B subunit, in which the combination of zinc with cholera vaccine and oral rehydration solutions (ORSs) has a positive impact on cholera and diarrhea [47].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 [48].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 [49]. 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 which many of the aforementined vaccine act on a single target, thus lacking a broad range of protection [50]. 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* [51]. 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 [52].

Table 2 exhibits Zn^{2+} ions binding vaccine activities for Gram-negative cell wall with outer membrane lipoprotein at C- and N-terminals and thin PGN layer in plasmic space.

Table 2. *Zn^{2+} ions binding vaccine activity of Zn^{2+} ions for Gram-negative cell wall with outer membrane lipoprotein and PGN layer in periplasmic space*

Zn^{2+}Ions	Gram-Negative Bacerial Cell Wall	
Zn^{2+} ions	• Membrane Lipoprotein at C- and N-terminals • Zn2+binding autolysins, Zn2+ions • Amidase gene amiB/LysM • Endopeptidase regulation • ETEC subunit vaccine • Oral vaccine by ORT • ZnuB against P. aeruginosa, • Preventive vaccine by recombinant flagella against P. aeruginosa	• PGN Thin Layer in Periplasmic Space • Zn2+binding autolysin, Zn2+ions • AmiC inPGN fragment release • Carboxypeptidase by transmission blocking vaccines • PGRPs or PGLYRPs • D-glutamate auxotrophy against P. aeruginosaPA14 • ORT in infectious diarrhoea Combination of zinc and choleravaccine and ORS • ZnuA against P. aeruginosa • Recombinant flagella and pili against P. aeruginosa
	• AfeA excellent vaccine antigen preventing infection of M.catarrhalis • Fusion DNA vaccine against Brucella abortus • ZnO-NPs disrupt the cell membrane and oxidative stress against Campylobacter	

ZnO Nanoparticles-Dependent Anti-Bacterial Vaccine

Zinc oxide (ZnO) nanoparticles (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 znd 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 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 peroxidation 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 in *Campylobacter*.

Conclusions

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. 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. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial vaccine activities. *S.aureus* amidase AmiA shed light 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 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. Major Atl autolysin also have an essential role in the early events of the FnBPs-dependent *S.aureus* biofilm phenotype.

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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. EVs are immuno-genic 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.

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. 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. Zinc has positive effect in children with complication 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 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 infecti-ous 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. 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 which many of the aforementined vaccine act on a single target, thus lacking a broad range of protection [50]. 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*. Multivalent fusion DNA vaccine against *Brucella abortus* has been constructed that the expression of BAB antigens conjugated to superoxide dismutase (SOD) protein can polarize mice immunity to a Th1-type phenotype, conferring low levels of protection in animal model.

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 znd 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.

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 they interact with biomolecules causing cell apoptosis leading to cell death. 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 in *Campylobacter*.

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