

## Bacteriolysis by Zinc Ions-Induced PGN Autolysins, Virucide by ZAP, ZBD, ZnONPs, and Prevailing Zinc Ions-Mediated HCoV Matter

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### ABSTRACT

Bacteriolysis by  $Zn^{2+}$ -induced peptidoglycan autolysins, virucide by zinc-finger antiviral protein (ZAP), zinc binding domain (ZBD), zinc oxide nanoparticles (ZnONPs), and current  $Zn^{2+}$  ions-mediated human coronavirus matter are respectively discussed. Bacterial peptidoglycan (PGN) autolysin AmiA for *S. aureus* amidase is acted on PGN binding and cleavage. The AmiA distinguishes PGN mostly by the peptide, and the cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against MRSA. 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- $\beta$ -(1,4)-MurNAc glycosidic bond of PGN building units. LytC, LytD, and LytF are expressed in the same subpopulation of cells and complete flagellar synthesis. Autolysin LytF is endopeptidase in *B. subtilis* that plays a role in cell separation and hydrolase of the peptide. Endopeptidase of autolysin LytF in *B. subtilis* plays a role in cell separation and hydrolase of the peptide. Endopeptidase of autolysin LytM is a glycyl-glycyl endopeptidase, hydrolyzing the pentaglycine interpeptide crossbridge. Thus,  $Zn^{2+}$  ions induced PGN autolysins for *S. aureus* is amidase LytA and endopeptidase LytM that are anticipated to be used as antibacterial potential of endogenous PGN-degrading enzymes against *S. aureus*.

Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth that zinc availability affects strong activity of cell wall hydrolases, and zur-regulated endopeptidases are present in divergent Gram-negative bacteria. AmiB catalyzes the degradation of PGN in Gram-negative bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. AmiC controls cell separation and PGN fragments release. Eps are predicted to hydrolyze PGN to facilitate cell growth that zinc availability affects strong activity of cell wall hydrolases. Eps at outer membrane lipoprotein and amidase, peptidase, and carboxypeptidase in PGN layer against *E. coli* are anticipated to be employed as *E. coli* cell wall-hydrolyzing enzymes of anti-bacterial potential. Zinc-dependent PGN autolysin of amidases are enhanced the anti-bacterial activities against both Gram-positive and Gram-negative bacteria. The autolysin-mediated bacteriolysis induced bacterial cell death can contribute to the bactericidal activities.

On the other hand enveloped viruses enter cells and initiate disease-causing cycles of replication in virus-cell interaction. The novel EBV-induced zinc finger gene ( $ZNF^{EB}$ ) including its intronless locus and human protein variants, controls virus entry and exit from cell cycling in activated lymphocytes. Zinc finger protein (ZNF) is prepared consisting HIV-1 type integrase fused to the synthetic zinc finger protein E2C that offers an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site. ZNF Tsip1 controls Cucumber mosaic virus (CMV) RNA replication. ZNF ZCCHC3 binds RNA and facilitates viral RNA that is critical for RIG-I like receptor (RLR)-mediated innate immune response to RNA virus.

ZAP inhibits entry, replication, and spread of certain viruses, and promotes viral RNA degradation. ZAP may regulate DNA and RNA virus replication that ZAP controls Retroviral RNA production and HIV-1 infection by promoting the degradation of specific viral mRNAs. ZAP-70 kinase regulates HIV cell-to-cell spread that HIV usurps components of the immunological synapse machinery to ensure its own spread through cell-to-cell contacts.

ZBD is essential for formation of the functional Junin virus envelope glycoprotein complex. ZBD inhibits Nidovirus RNA synthesis and replication, hence the 2019-nCoV may be regulated by ZBD.

ZnONPs recently are used in various applications of veterinary science due to their antibacterial and antiviral agents, tissue repair that the ZnONPs are anticipated to be employed in prevention of human coronavirus infection.

Today, seventh known HCoVs have been identified, namely HCoVs-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and recently new-typed 2019-nCoV, subsequent phylogenetic studies pointed to the bat origin of SARS-CoV based on sequences of SARS-like virus found in bats. This 2019-nCoV ( $\beta$ -CoV) structure has a spike glycoprotein (S) that the coronavirus protein mediates coronavirus entry into host cells which the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion must be considered to be able to degrade or suppress for the spikes and the membrane by  $Zn^{2+}$ -centered tetrahedrally coordinated binding.

Anti-viral activities of  $Zn^{2+}$  ions released from ZNF, ZAP, ZBD, and ZnONPs are recognized by which highly diverse fusion proteins have converged on the same overall strategy to mediate a common pathway of membrane fusion, Zinc ion drug development is anticipated to be adopted by using ZAP viral destruction via cell surface receptors and  $Zn^{2+}$ -coordination pattern, causing to lead enhancement of the anti-viral activity.

**Keywords:** Bacterial PGN autolysin, Autolysin amidase, Zinc-finger antiviral protein, Virus entry, replication and spread, Zinc oxide nanoparticles, Human Coronavirus, Prevention of respiratory and pneumonic spreading

**Abbreviations;** Aas= auto lysin/ adhesin of *Staphylococcus saprophyticus*, ABC=ATP-binding cassette, APC=antigen presenting cell, *A. stephensi*= *Anopheles stephensi*, *B. abortus*= *Brucella abortus*, *B. subtilis*=*Bacillus subtilis*, CBDs=cell wall binding domains, CBPs=choline binding proteins, *C. difficile*= *Clostridium difficile*, CKD=Chronic Kidney Disease, CMV=Cucumber mosaic virus, *E. coli*=*Escherichia coli*, *E. faecalis*= *Enterococcus faecalis*, ETEC=Enterotoxigenic *E.coli*, *Eps*=Zinc dependent endopeptidases, *FnBPs*= fibronectin-binding proteins, Gas=group A streptococcus, GelE=gelatinase, HCoV=human coronavirus, HCV=hepatitis C virus, HD=hemodialysis, HIV-1=Human immunodeficiency virus type 1, IFITMs=Interferon transmembrane proteins, *M. catarrhalis*=*Moraxella catarrhalis*, MCPs= M et allocarboxypeptidases, MIBRs= most probable immunoprotective B-cell epitope regions, MRB= multidrug bacteria, 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, RdRp= **RNA-dependent RNA polymerase**, RIG-1=retinoic acid-inducible gene-1, RLR=RIG-1 like receptor, ROS=reactive oxygen species, Sags= super-antigens, SasG=*S. aureus* surface protein, *S. aureus*=*Staphylococcus aureus*, SBP= solute-binding protein, SEB=staphylococcal enterotoxin serotype B, SINV=Sindbis virus, SOD= superoxide dismutase, *S. pneumoniae*= *Streptococcus pneumoniae*, SSP=stable signal peptide, TBVs= transmission-blocking vaccines, Tsi1= Tsi1-interacting protein 1, VRE=vancomycin-resistant *Enterococcus faecium*, ZAP=zinc-finger antiviral protein, ZBD=zinc-binding domain, ZBL=zinc binding lipoprotein, ZNF<sup>EB</sup>=EBV-induced zinc finger gene, ZnONPs=zinc oxide nanoparticles, ZnuA=Zinc uptake A.

## INTRODUCTION

Zinc is a nutritionally fundamental trace element in human body that the recommended daily intake of zinc depends on several factors. Average values of recommended intake may be 7-11 mg/day for adults. Zinc deficiency currently accounts for approximately 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrheal diseases, while severe zinc deficiency is rare, mild to moderate deficiency is more common worldwide [1]. Zinc homeostasis is a key factor in maintaining a healthy immune system that 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 reactive nitrogen species (RNS) [1]. The other, zinc excess provokes an impairment of the immune system and has significant toxicity to bacteria.

Zinc is known to be essential for highly growth and development of all organisms in the human body, especially the immune system. A variety of effects of zinc on immune cells depend on the zinc concentration that in a concentration of 100 µmol/L, zinc suppresses natural killer cell killing and T-cell function whereas monocytes are activated directly, and in a concentration of 500 µmol/L, zinc evokes a direct chemotactic activation of neutrophil granulocytes [2]. The zinc ions play important roles of effects on prevention and reduction for bacterial and viral infections. Bacteria and viruses are most common cause of foodborne disease by food poisoning and many human illnesses are caused by bacterial and viral infections. Bacterial and viral infections are associated with deficiencies in macronutrients and micronutrients, including the essential trace elements that dietary supplementation to provide adequate element supply has been proposed to confer health benefits for patients suffering from some bacterial or viral diseases [3]. The treatments with herbal nutraceuticals and zinc likely indirectly contributed to the increase in the resistance of the lambs against *Haemonchus contortus* infection [4]. Zinc is the second most abundant trace metal with human body 2-3g and a plasma concentration of 12-16 µM, 90% in muscle and bone, and 10% other organs include prostate, liver, the gastrointestinal tract, kidney, skin, lung brain, heart, and pancreas in humans that cellular zinc underlies an efficient homeostatic control that avoids accumulation of zinc in excess. 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 [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 endogenous death in the infected cell, and super antigens [6]. Zinc influences apoptosis by acting on several molecular regulators of programmed cell death and zinc deficiency caused by malnutrition and foods with low bio-

availability, aging, certain diseases, and deregulated homeostasis is a far more common risk to human health without intoxication [7]. The influence of zinc on apoptosis is tissue/cell type, zinc concentration, and expression of zinc transporters and zinc-binding proteins.

The other, zinc deficiency in Chronic Kidney Disease (CKD) patients may be due to fecal excretion or decrease in its absorption that zinc concentrations were lower in hemodialysis (HD) patients compared to controls and Zn concentration 69.16 µg/dL of blood in HD patients, however, revealed no correlation among serum Zn concentration and anemia, serum parathyroid hormone concentration or pruritus severity in HD patients [8].

Bacterial killing of Zn<sup>2+</sup> ions occurs chiefly by bacteriolyses of bacterial cell walls due to activated peptidoglycan (PGN) autolysins such as amidases, endopeptidases, and carboxypeptidase against bacteria [9]. 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 [10]. 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 [11].

Viruses are intracellular obligate parasites that cause infection by invading cells of the body. Their life cycle comprises a short extracellular period and a longer intracellular period during which they undergo replication. The immune system has non-specific and specific mechanism that attacks the virus in both phases of its life cycle which specific antibodies protect against viral infections and play an important role in antiviral immunity, mainly during the early stage of the infection [12]. To date, there are presence as severe problems in the world against the infection of new-typed human coronavirus (HCoV) of 2019-novelCoV. The rapid final completion is anticipated.

In this review, firstly, bacteriolysis of bacterial cell walls by Zn<sup>2+</sup> ions induced autolytic PGN activation are debated against *Staphylococcus*

*aureus* (*S. aureus*) as Gram-positive bacterium and *Escherichia coli* (*E. coli*) as Gram-negative bacterium. Secondly, the zinc-mediated antiviral immunity and viral inhibition by zinc-finger protein, zinc-finger antiviral protein, zinc-binding domain, and zinc oxide nanoparticles are discussed, and Zn<sup>2+</sup> ions-mediated antiviral against Human Coronaviruses also is dealt with. Lastly, the bacteriolytic and the virucidal mechanisms on the bacteriolysis by Zn<sup>2+</sup> ions-induced PGN autolytic activation and on the ZAP-induced virucide strain are respectively clarified.

### **ZINC-INDUCED BACTERIAL AND VIRAL IMMUNITY TO MAINTAIN CONSTANT HOMEOSTASIS**

Zinc-induced anti-bacterial immunity is important factor to be both essential and toxic for microorganism that zinc (Zn<sup>2+</sup>) ions have crucial roles in many facets of the immune system, in which microbial susceptibility to Zn(II) toxicity is mediated by extracellular cation competition and this susceptibility can be harnessed by innate immune response [13].

It is essential for bacteria to maintain metal ion homeostasis that the need for tight homeostatic control is particularly true for zinc, an essential transition metal ion which zinc ions may be used as an antimicrobial agent in the innate immune system and zinc efflux is an important contributor to group A *Streptococcus* (GAS) pathogenesis [14]. Maintaining a constant state of cellular zinc homeostasis is essential for normal function that typically, human zinc intakes range from 107 to 231 µmol/dm<sup>3</sup>; this is equivalent to 14 ~ 30 mg/kg for comparison with rat diets [15]. Zinc-modulating immune response depends on a sufficient availability of this zinc that zinc supplementation in diseases diarrhea, chronic hepatitis pneumonia, and acute lower respiratory infection seems beneficial [16]. Zinc induced antiviral immunity plays an important role in antiviral antibodies that zinc binds to the viral envelope or capsid proteins, and block the virus from entering into host cell, in which cytotoxic T lymphocytes (CTL) cells in specific antiviral immunity recognize viral antigens presented at the cell surface associated with I major histocompatibility complex (MHC) molecule [17]. An essential trace element zinc is crucial for growth, development, and the maintenance of immune function which zinc status is a critical factor that can influence antiviral immunity, particularly as zinc-deficient



populations are often most at risk of acquiring viral infections such as HIV, HCV [18]. Common features possess that enveloped viruses enter cells by membrane-fusion protein on the surface, fusion glycoprotein on metastable prefusion and interactions with neutralizing antibodies. Implications for immunogen design of next-generation vaccines have been shown from the results that stable immunogens presenting the same antigenic sites as the labile wild-type proteins efficiently elicit potentially neutralizing antibodies [19].

### **BACTERIOLYSES BY $Zn^{2+}$ ION-INDUCED PGN AUTOLYSINS**

#### **Molecular Structures of *S. Aureus* and *E. coli* Cell Walls and the Action Sites of PGN Autolysins**

Bacterial PGN structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and  $\beta$ -(1-4)-N-acetylmuramic acid (NAM) that are crosslinked by peptide stem chains attached to the NAM residues [20]. The action sites of bacterial autolysins are comprised that for *Staphylococcus aureus* (*S. aureus*) PGN layer cell wall, there are N-acetylmuramidase-L-alanine amidase and PGN chain cross-linkage DD-endopeptidase.

The other, for *Escherichia coli* (*E. coli*) cell wall, there are endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals, and amidase, peptidase, and carboxypeptidase at thin PGN layer in periplasmic space [21]. 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 hydrolases of amidase, glycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [22]. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial activities.

#### **$Zn^{2+}$ Ions Induced Activated PGN Autolysins Promote the Bacteriolysis against Gram-Positive Bacterial Cell Wall**

*S.aureus* amidase AmiA is acted on PGN binding and cleavage. The AmiA distinguishes

PGN mostly by the peptide, and the cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against MRSA [23]. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of *Staphylococcus saprophyticus*) is inhibited and is necessary for the C-terminal GW repeats, not the N-terminal repeats [24].

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 [25]. 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 [26]. The LytB PGN hydrolase responsible for physical separation of daughter cells cleaves the GlcNAc- $\beta$ -(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 [27]. The PGN-remodeling autolysins LytC, LytD, and LytF are expressed in the same subpopulation of cells and complete flagellar synthesis that LytC appears to be important for flagellar function, motility was restored to a LytC mutant by mutation of either lon A, and LytC, LytD, and LytF autolysins to population heterogeneity in *B. subtilis* [28].

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 [29]. The biochemical and structural staphylococcal Atl have successful cloning, high level over-expression, and purification Atl proteins [30]. AtlA is the major PGN hydrolases of *Enterococcus 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 cleavage event [31]. Major Atl autolysin also have an essential role in the early events of the

fibronectin-binding proteins (FnBPs)-dependent *S.aureus* biofilm phenotype [32]. For the contribution of autolysins of PGN hydrolases to bacterial killing, there are N-acetyl-glucosaminidase (At1A), two N-acetyl-muraminases (At1B and At1C) [33].

Endopeptidase of autolysin LytF in *B. subtilis* plays a role in cell separation and hydrolase of the peptide [34]. Endopeptidase of autolysin LytM is a glycyl-glycyl endopeptidase, hydrolyzing the pentaglycine interpeptide crossbridge [35].

Thus, Zn<sup>2+</sup> ions induced PGN autolysins for *S. aureus* is amidase LytA and endopeptidase LytM that are anticipated to be used as antibacterial potential of endogenous PGN-degrading enzymes against *S. aureus* [36].

**Zn<sup>2+</sup> Ions Induced Degrading Enzyme of Outer Membrane Lipoprotein and PGN Autolysins Promote the Bacteriolysis against Gram-Negative Bacterial Cell Wall**

Zinc-dependent endopeptidases (Eps) are predicted to hydrolyze PGN to facilitate cell growth that zinc availability affects strong activity of cell wall hydrolases, and zinc-regulated endopeptidases are present in divergent Gram-negative bacteria [37].

Amidase gene (AmiB) catalyzes the degradation of PGN in Gram-negative 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 division and inactivation of the amiB gene, resulting in a marked increase of sensitivity to oxidative stress and organic acids [38]. Amidase activity of amiC controls cell separation and PGN fragments release [39].

Zinc-regulated peptidase maintains cell wall integrity during immune-mediated nutrient sequestration against *Acinetobacter baumannii* [40].

Carboxypeptidases are exopeptidases that remove a single amino acid residue from the C terminus of proteins or 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 transmission-blocking vaccines (TBVs) against Malaria eradication [41]. Metallo-carboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against *Trypanosoma brucei* or *cruzi* [42]. Zinc-dependent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial activities. Thus, Endopeptidase at outer membrane lipoprotein and amidase, peptidase, and carboxypeptidase in PGN layer against *E. coli* are anticipated to be employed as *E. coli* cell wall-hydrolyzing enzymes of antibacterial potential.

Human peptidoglycan recognition proteins (PGRPs) are novel class of recognition and effector molecules with broad Zn<sup>2+</sup>-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria [43]. Hence, zinc-dependent PGN autolysin of amidases are enhanced the anti-bacterial activities against both Gram-positive and Gram-negative bacteria. Thus, autolysin-mediated bacteriolysis induced bacterial cell death can contribute to the bactericidal activities. Further, autolysin-mediated lysis-induced bacterial cell death may make contribution to the bacteriolytic vaccine activities.

Accordingly, anti-bacterial activities of bacteriolyses by Zn<sup>2+</sup> ions induced activated PGN autolysins against *Gram-positive* thick PGN layer, and Gram-negative outer membrane lipoprotein and thin PGN layer cell walls are represented in **Table 1**.

**Table 1.** Zinc induced bacteriolysis against Gram-positive thick PGN envelope cell wall and Gram-negative lipoprotein and thin PGN layer cell wall

Zn <sup>2+</sup> Ions	Gram-Positive PGN Layer Cell Wall
Zn <sup>2+</sup>	<p>→ Zn<sup>2+</sup>, O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, ·OH, ·NO, ONOO<sup>-</sup></p> <p><b>Zn<sup>2+</sup> ions induced activated PGN autolysins</b></p> <ul style="list-style-type: none"> <li>• <i>S.aureus</i> amidase AmiA</li> <li>• Recombinant amidase of the <i>Aas</i></li> <li>• Lytic amidase LytA for <i>Streptococcus pneumoniae</i></li> <li>• Pneumococcal autolysin LytA, LytC, D, F of PGN remodeling for <i>Bacillus subtilis</i></li> <li>• Endopeptidase LytF for <i>bacillus subtilis</i></li> <li>• At1A autolysin for GelE against <i>E. faecalis</i></li> <li>• At1A, At1B, At1C autolysins against <i>enterococcus faecalis</i></li> <li>• Fusion protein autolysin, MIBRs against <i>S. pneumoniae</i></li> <li>• Metallo-carboxypeptidase M32 against <i>Trypanosoma brucei</i> or <i>cruzi</i></li> <li>• PBP2a and autolysin mixture against <i>MRSA</i></li> </ul>

Zn <sup>2+</sup> ions	Gram-Negative Cell Wall	
Zn <sup>2+</sup>	Outer Membrane Lipoprotein at C- and N-terminals	Periplasmic Space Thin PGN Layer
	<p>→ Zn<sup>2+</sup>, O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub></p> <ul style="list-style-type: none"> <li>• Amidase gene <i>amiB</i>/LysM</li> <li>• Endopeptidase regulation of ShyA and ShyB</li> <li>• Outer membrane receptor against <i>N.menigitidis</i></li> <li>• ETEC subunit vaccine</li> <li>• ZnuB against <i>P. aeruginosa</i>.</li> <li>• Preventive vaccine by recombinant flagella against <i>P. aeruginosa</i></li> </ul>	<p>→ Zn<sup>2+</sup>, O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, OH<sup>-</sup>, ·OH</p> <ul style="list-style-type: none"> <li>• AmiC in PGN fragment release</li> <li>• Carboxypeptidase by transmission-blocking vaccines</li> <li>• PGRPs have Zn<sup>2+</sup>-dependent bactericidal activity</li> <li>• D-glutamate auxotrophy against <i>P. aeruginosa</i> PA14</li> <li>• Zn in infectious <i>diarrhoea</i></li> <li>• <i>ZnuA</i> against <i>P. aeruginosa</i></li> <li>• Recombinant flagella and pili against <i>P. aeruginosa</i></li> <li>• Carboxypeptidase B1 against <i>Anopheles stephensi</i> and for malaria as transmission- blocking</li> </ul>

### VIRUCIDAL ACTIVITIES OF ZINC-FINGER ANTIVIRAL PROTEIN, ZINC-BINDING DOMAIN, AND ZINC OXIDE NANOPARTICLES

#### Zinc-Finger Protein

Zinc salts (Zn<sup>2+</sup> concentration: < 80 μM) suppressing the activity of viral RNA-dependent RNA polymerase, brock hepatitis E virus (HEV) replication [44]. Respiratory syncytial virus (RSV) is the most important viral cause of acute respiratory tract infection (ARI) that the complete inhibitory effect of zinc salts (Zn<sup>2+</sup> concentration: 1 and 10 mM) on RSV plaque formation was observed, in which zinc (+) ions mediate antiviral activity on RSV by altering the ability of the cell to support RSV replication [45].

The novel EBV-induced zinc finger gene (*ZNF<sup>EB</sup>*) including its intronless locus and human protein variants, controls entry and exit from cell cycling in activated lymphocytes [46]. The designed polydactyl zinc-finger protein (ZNF) is prepared consiting HIV-1 type integrase fused to the synthetic zinc finger protein E2C that offers an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site [47]. The ZNF ZCCHC3 binds RNA and facilitates viral RNA that ZCCHC3 is a co-receptor for the retinoic acid-inducible gene-1 (RIG-1) and antigen MDA5 which is critical for RIG-1 like receptor (RLR)-mediated innate immune response to RNA virus [48]. Artificial ZFN were targeted to the high affinity Sp1-binding site, and by being

fused with TatdMt and POZ domain, they strongly block both Sp1-cyclin T1-dependent transcription and Tat-dependent transcription of HIV-1 [49]. ZNF Tsi1 that the candidate genes encoded Tsi1-interacting protein 1 (Tsi1), a zinc (Zn) finger protein Tsi1 strongly interacted with CMV 2a protein, controls Cucumber mosaic virus (CMV) RNA replication [50].

#### Zinc-Finger Antiviral Protein

The zinc-finger antiviral protein (ZAP) controls virus entry, DNA/RNA replication, and spreading against viral infection. The ZAP in first steps of HCV infection may be used as entry inhibitor [51]. Interferon induced transmembrane proteins (IFITMs) inhibit the cellular entry of a broad range of viruses that IFITM-mediated restriction requires recognition of viral RNA elements [52]. The interferon-stimulated genes serve as enhancers of antiviral innate immunity [53]. ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism by which ZAP inhibits Sindbis virus (SINV) translation may lead to the development of agents with broad activity against alphaviruses [54]. The ZAP also inhibits Influenza A virus (IAV) protein expression, in which suggests an important role of ZAP in the host effort to control IAV infection and the importance of the threat of ZAP to the virus [55]. The host cell restriction factors that limit IAV have been investigated [56]. ZAP may regulate DNA and RNA virus replication. Inhibition of bacterial DNA replication during

nitrosative stress is accompanied by zinc mobilization [57]. ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation [58]. ZAP inhibits Retroviral RNA production [59] and ZAP inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs [60].

### **ZAP Regulates Spread**

ZAP' stress with antiviral activity and induced virus replication are regulated upon virus infection to inhibit virus spread [61]. ZAP-70 kinase regulates HIV cell-to-cell spread that HIV usurps components of the immunological synapse machinery to ensure its own spread through cell-to-cell contacts [62]. An understanding of viral cell-to-cell transmission spreading will enhance our ability to intervene in the efficient spreading of viral infection [63].

### **Zinc-Binding Domain**

A novel zinc-binding domain (ZBD) is essential for formation of the functional Junin virus envelope glycoprotein complex that the envelope glycoprotein of the Junin arenavirus (GP-C) mediates entry into target cells through a pH-dependent membrane fusion mechanism, in which this unusual motif may act to retain a cleaved 58-amino-acid stable signal peptide (SSP) for its role in modulating membrane fusion activity [64]. Entry of the virus into the host cell is mediated by the viral envelope glycoprotein, GPC that SSP was retained in GPC through interaction with a zinc-binding domain (ZBD) in the cytoplasmic tail of transmembrane fusion of G2 subunits that Junin virus ZBD displays a novel fold containing two zinc ions, in which the structural basis for retention of the unique SSP submit suggests a mechanism whereby SSP is positioned in the GPC complex to modulate pH-dependent membrane fusion [65]. In addition, ZBD inhibits Nidovirus RNA synthesis and replication [66], hence the 2019-nCoV may be regulated by ZBD.

### **Viral Membrane Fusion Protein**

Enveloped viruses enter cells and initiate disease-causing cycles of replication that in all cases virus-cell fusion is executed by one or more viral surface glycoproteins denoted as the fusion protein, in which the structure and mechanisms on viral membrane fusion protein are important problems [67]. The membrane fusion reaction, membrane interaction, conformational changes of specialized virus

envelope proteins, and refolding reactions of specific fusion proteins can mediate both virus-cell fusion leading to infection and pathological cell-cell fusion, in which they are increasingly viewed as targets for antiviral intervention [67].

### **Zinc Oxide and ZnONPs Inhibit Virus Entry, Replication, and Spread**

Zinc oxide tetrapods (ZnOTs) of micro-nanostructures block HSV-2 attachment, entry into host cell, and stop the spread of the virus among already infected cells [68]. ZnOTs can inhibit HSV-1 growth and spread in corneal tissues, hence, the ZnOTs also could control HCoVs [69].

Zinc has broad-spectrum antiviral activity against HIV, transmissible gastroenteritis virus (TGEV), and SARS-CoV that 2,500 mg/kg diet Zn<sup>high</sup> showed a down-regulation of interferon (IFN)- $\alpha$ , oligoadenylate synthetase (OAS), Zn transporter (ZIP4), as well as the Zn transporters (ZnT1) and (ZnT5), in which the Zn<sup>high</sup> documented an earlier and higher systematic TGEV-specific serum antibody response [70].

Zinc oxide nanoparticles (ZnONPs) recently are used in various applications of veterinary science due to their antibacterial and antiviral agents, tissue repair that the ZnONPs are anticipated to be employed in prevention of human coronavirus infection. Firstly, ZnONPs inhibit H1N1 influenza virus entry into the host cells [71]. ZnONPs-5  $\mu$ g/ml specifically regulated the correlation of microRNAs and their targeted genes [72]. Zinc oxide nanoparticles to dimethylnitrosamine (DMN) treated rats inhibit the production of mRNA of inflammatory cytokines and reduce lipid peroxidation, oxidative stress and fibrosis in the liver [73].

### **Zinc Induced Human Coronavirus and Prevention of Respiratory Ailment and Pneumonia against HCoVs**

Coronaviruses (CoVs) and arteriviruses are related human and animal pathogens that belong to the Coronaviridae family in the order Nidovirales that CoVs have the largest known RNA genomes. The RNA-dependent RNA polymerase (RdRp) and enzymatic functions for nidoviruses that are characterized by polycistronic plus-stranded RNA genome are required to suppress the consequences of the typically high error rate of viral RdRps that the RdRp behaviour and interactions during RNA synthesis, and subsequent processing must be



understandable [74]. Zinc ions are essential for the rescue of the enzymatic activities of nidovirus helicases that a complex zinc finger can inhibit possibly virion biogenesis, nidovirus replication and transcription, and disrupting RNA synthesis [66].

Human coronaviruses (HCoVs) are recognised as coronaviruses (CoVs) associated with multiple respiratory diseases of varying severity, including common cold, pneumonia and bronchitis that to date, seventh known HCoVs have been identified, namely HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and recently new-typed 2019-nCoV, subsequent phylogenetic studies pointed to the bat origin of SARS-CoV based on sequences of SARS-like virus found in bats [75]. The new developed drugs for the 2019-nCoV has been now reported that Lopinavir/ Ritonavir, Nucleoside analogues, Neuraminidase inhibitors, Remdesivir, peptide (EK1), abidol, RNA synthesis inhibitors (such as TDF, 3TC), anti-inflammatory drugs (such as hormones and other molecules), Chinese traditional medicine, such Shu Feng JieDu Capsules and Lianhuaqingwen Capsule, could be the drug treatment options for 2019-nCoV. However, the efficacy and safety of these drugs for 2019-nCoV still need to be further confirmed by clinical experiments [76]. HCoVs are a well-known cause of respiratory infections, in which  $Zn^{2+}$  inhibits coronavirus and arterivirus RNA polymerase activity, and zinc ionophores block the virus replication that the combination of  $Zn^{2+}$  and pyrithione at low concentrations ( $Zn^{2+}$  concentration 2  $\mu M$ ) inhibits the replication of SARS-CoV and arterivirus RNA [77]. High zinc ion concentration (10 and 100  $\mu M$ ) to the addition of compounds and Zn concentration increasing in Cu/Zn brasses were found to inhibit the replication of various RNA virus, influenza viruses, respiratory syncytial virus and human coronavirus 229E [78].

Replication of SARS-CoV requires proteolytic processing of the replicase polyprotein by two viral cysteine proteases, a chymotrypsin-like protease (3CLpro) and a papain-like protease (PLpro). This PLpro is important for development of antiviral drug that would inhibit viral replication and reduce mortality associated with outbreaks of SARS-CoV that a model of PLpro in complex with ubiquitin reveals well

defined sites within the catalytic cleft that help to account for strict substrate-recognition motifs [79]. The MERS-CoV PLpro blocking loop 2 (BL2) structure differs from that of SARS-CoV PLpro, where it has been proven to play a crucial role in SARS-CoV PLpro inhibitor binding that inhibitor recognition specificity of MERS-CoV PLpro may differ from that of SARS-CoV PLpro. Inhibitory activity, of this compound was selective for SARS-CoV and MERS-CoV PLpro enzymes over two human homologues, and the ubiquitin C-terminal hydrolases [80]. The papain-like protease 1 (PL1<sup>pro</sup>) domain is present in nonstructural protein 3 (nsp3) of alpha coronavirus and subgroup 2a beta coronavirus, and the papain-like protease 2 (PL2<sup>pro</sup>) is present in SARS-CoV. In combination with the prior characterization of PL2pro from other alpha coronavirus of human coronavirus 229E, NL63, these viruses employ two PL<sup>pro</sup>s with overlapping specificities toward both viral and cellular substrates [81]. ZAPs also could probably inhibit the HCoVs that the ZAP could regulate RNA virus degradation of SARS-CoV's and MERS-CoV's RNA virus.  $Zn^{2+}$  ions are capable of inhibiting PLpro activity and the zinc conjugates to inhibit SARS-CoV PLpro activity that targeting PLpro with antiviral drug may have an advantage in not only inhibiting viral replication but also inhibiting the dys-regulation of signaling cascades infected cells, leading to cell death [82]. Further, zinc conjugated complexes as SARS-CoV 3C-like protease inhibitors play important role for this  $Zn^{2+}$ -centered coordination pattern that the zinc-coordinating inhibitor is tetrahedrally coordinated to the His<sup>40</sup>-Cys<sup>147</sup> catalytic dyad of CVB3 3C<sup>pro</sup> [83, 84].

Most of the coronaviruses can cause the infectious diseases in human, currently, there are four coronaviruses:  $\alpha$ -CoV,  $\beta$ -CoV,  $\gamma$ -CoV, and  $\delta$ -CoV that  $\alpha$ -CoV and  $\beta$ -CoV mainly infect the respiratory, gastrointestinal, and central nervous system of humans and mammals, while  $\gamma$ -CoV and  $\delta$ -CoV mainly infect the birds, in which 2019-nCoV or SARS-CoV-2 belongs to the  $\beta$ -CoV according to the phylogenetic analysis based on the viral genome [85]. This  $\beta$ -CoV structure has a spike glycoprotein (S) that the coronavirus protein mediates coronavirus entry into host cells which the evolution of these two critical function of coronavirus spike proteins, receptor recognition and membrane fusion must be considered [86]. It is responsible for binding



## Bacteriolysis by Zinc Ions-Induced PGN Autolysins, Virucide by ZAP, ZBD, ZnONPs, and Prevailing Zinc Ions-Mediated HCoV's Matter

to the receptor on the host cell as well as mediating the fusion of host and viral membrane, including with a process driven by major conformational changes of the S protein [87]. Zinc ion drug development is anticipated to be adopted by using ZAP Viral destruction via cell surface receptors [88] and  $Zn^{2+}$ -coordination pattern [89]. Zinc ions may be functioned with the viral spike(S), envelope (E), nucleocapsid (N) proteins, and membrane (M) in human coronavirus particles by  $Zn^{2+}$ -centered tetrahedrally coordinated binding to these microproteins. Zinc is efficient for the prevention of respiratory ailment and pneumonia that the N protein of HCoV induces aggregation of human elongation factor 1-alpha, inhibiting protein translation and cytokinesis by blocking filamentous-actin bundling and proliferation of several steps in T-lymphocyte

generation was significantly inhibited by the infection of recombinant retrovirus expressing HCoV N protein, in which there will result in zinc ions-induced prevention of infectious and pneumonic spreading [90]. Accordingly, appreciable  $Zn^{2+}$  ion solutions may be most effective for the prevention of coronavirus infection, subsequent respiratory illness that the lower  $Zn^{2+}$  ion concentration ( $< 80 \mu M$ ) could be efficient for vaccine candidates and the higher  $Zn^{2+}$  ion concentration ( $\approx 100 \mu M$ ) should be available for the pulmonary disease and the infectious spreading.

As mentioned above, the virucidal activities of ZNF, ZAP, ZBD, ZnO-NPs and prevention of coronavirus infection and respiratory ailment for virus entry, replication, and spread are represented in Table 2.

**Table2.** Virucidal activities of ZNF, ZAP, ZBD, and ZnONPs for prevention, virus entry, replication, and spread against CMV, HCV, HEV, HIV, MLV, and HCoVs

$Zn^{2+}$ ions	Virucidal activities of $Zn^{2+}$ from ZNF, ZAP, ZBD, and ZnONPs for prevention, virus entry, replication, and spread against CMV, HCV, HEV, HIV, MLV, and HCoVs	
$Zn^{2+}$	<b>Adsorption/Entry</b>	<b>DNA/RNA Replication, Spread</b>
	<p>→ <math>Zn^{2+}</math>, <math>\cdot O_2^-</math>, <math>H_2O_2</math></p> <ul style="list-style-type: none"> <li>• EBV-induced zinc finger gene <math>ZNF^{EB}</math> controls entry and exit</li> <li>• ZBD prevent viral entry and and GPC inhibit activate membrane fusion</li> <li>• Zn-metalloprotease inhibits entry and cell-cell fusion</li> <li>• ZnOTs block HSV-2 attachment</li> <li>• ZnOTs inhibit HSV-1 entry and spread</li> <li>• 2,500mg/kg diet ZnO has antiviral activity of SARS-CoV</li> <li>• ZnONPs inhibit H1N1 influenza virus entry</li> <li>• Lower <math>Zn^{2+}</math> conc may be efficient for vaccine candidate and higher <math>Zn^{2+}</math> conc may prevent respiratory ailment and acute pneumonia spreading against HCoVs</li> </ul>	<p>→ <math>Zn^{2+}</math>, <math>\cdot O_2^-</math>, <math>H_2O_2</math>, NO</p> <ul style="list-style-type: none"> <li>• Zinc salts inhibit HEV RNA replication</li> <li>• ZAP inhibits entry of Sindbis virus, HCV</li> <li>• IFTMs as cellular entry inhibitor</li> <li>• ZAP inhibits replication of MLV</li> <li>• ZAP-mediated RNA degradation</li> <li>• Zinc finger: virus decay</li> <li>• Zinc finger proteinE2C; viral DNA specific sites</li> <li>• Zinc finger protein Tsp1;Cucumber mosaic virus(CMV)RNA replication</li> <li>• Artificial zinc finger fusion; HIV-1 tanscriptions</li> <li>• ZAP inhibits HIV, SINV spread</li> <li>• ZnONPs regulate microRNA in Ovarian granulosa cells</li> <li>• ZnONPs + DMN inhibit the production of mRNA of inflammatory cytokines</li> <li>• ZAP degrades SARS-CoV's and MERS-CoV's RNA</li> <li>• Complex zinc-finger inhibits nidovirus replication</li> </ul>

## CONCLUSIONS

Bacteriolyses by  $Zn^{2+}$  ions-induced activated PGN autolysins, virucides by ZAP, ZBD, and ZnONPs, and prevalent human coronaviruses

have been discussed, and the bacteriolytic and virucidal mechanisms are clarified respectively.

Bacterial peptidoglycan (PGN) autolysin AmiA for *S.aureus* amidase is acted on PGN binding

and cleavage. AmiA distinguishes PGN mostly by the peptide, and the 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 necessary for the C-terminal GW repeats.

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- $\beta$ -(1,4)-MurNAc glycosidic bond of PGN building units. LytC, LytD, and LytF are expressed in the same subpopulation of cells and complete flagellar synthesis.

Ami B catalyzes the degradation of PGN in Gram-negative bacteria, resulting in a marked increase of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell separation and PGN fragments release. Enterotoxigenic *E. coli* (ETEC) is the most common bacterial cause of children's diarrhea, in which antigen and antitoxin antibodies that neutralized both toxins that are associated with all cases of ETEC diarrhea. 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 activities, where PGN autolysins interact with biomolecules causing cell apoptosis leading to cell death. Human peptidoglycan recognition proteins (PGRPs) are novel class of recognition and effector molecules with broad Zn<sup>2+</sup>-dependent bactericidal activity against both Gram-positive and Gram-negative bacteria. In these autolysins, amidases of zinc-dependent PGN autolysin are enhanced and induced the anti-bacterial activities against bacterial infection. Thus, autolysin-mediated bacteriolysis - induced bacterial cell death can contribute to the bactericidal activities.

On the other hand, enveloped viruses enter cells and initiate disease-causing cycles of replication that in all cases virus-cell fusion is executed by one or more viral surface glycoproteins denoted as the fusion protein. The novel EBV-induced zinc finger gene (*ZNF<sup>EB</sup>*) controls entry and exit from cell cycling in activated lymphocytes. The designed polydactyl ZNF is prepared consisting HIV-1 type integrase fused to the synthetic zinc finger protein E2C. The zinc-finger antiviral

protein (ZAP) specifically inhibits virus entry, replication, and spread of certain viruses, and ZAP inhibits the replication of certain viruses and promotes ZAP-mediated viral RNA degradation. The ZAP also inhibits Influenza A virus (IAV) protein expression. Furthermore, ZAP inhibits Retroviral RNA production and inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs. ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation.

ZAP' stress with antiviral activity and induced virus replication are regulated upon virus infection to inhibit virus spread. ZAP-70 kinase regulates HIV cell-to-cell spread that HIV usurps the immunological components to ensure its own spread through cell-to-cell contacts.

A novel zinc-binding domain (ZBD) is essential for formation of the functional Junin virus envelope glycoprotein complex.

Thus, ZNF, ZAP, and ZBD specifically inhibit virus entry, replication, and spread of many viruses. The host-virus interaction, conformational changes of specialized virus envelope proteins, and refolding reactions of specific fusion proteins in an essential steps entry, replication, and spread of enveloped virus life cycle have been worthy of remark in fascination that these diverse viral fusion protein could be used in next-generation for therapeutic intervention in arenaviral disease.

Zinc oxide and ZnONPs inhibit virus entry, replication, and spread. Zinc oxide tetrapods (ZnOTs) of micro-nanostructures block HSV-2 attachment, entry into host cell, and stop the spread of the virus among already infected cells. ZnONPs recently are used in various applications of veterinary science due to their antibacterial and antiviral agents, tissue repair that the ZnONPs are anticipated to be employed in prevention of human coronavirus infection. ZnONPs-5 mg/ml specifically regulated the correlation of microRNAs and their targeted genes. ZnONPs to dimethylnitrosamine (DMN) treated rats inhibit the production of mRNA of inflammatory cytokines and reduce lipid peroxidation, oxidative stress and fibrosis in the liver.

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His<sup>40</sup>-Cys<sup>147</sup> catalytic dyad of CVB3 3C<sup>pro</sup>. Most of the coronaviruses can cause the infectious diseases in human, currently, there are four coronaviruses:  $\alpha$ -CoV,  $\beta$ -CoV,  $\gamma$ -CoV, and  $\delta$ -CoV that  $\alpha$ -CoV and  $\beta$ -CoV mainly infect the respiratory, gastrointestinal, and central nervous system of humans and mammals, while  $\gamma$ -CoV and  $\delta$ -CoV mainly infect the birds, in which 2019-nCoV or SARS-CoV-2 belongs to the  $\beta$ -CoV according to the phylogenetic analysis based on the viral genome. This  $\beta$ -CoV structure has a spike glycoprotein (S) that the coronavirus protein mediates coronavirus entry into host cells which the evolution of these two critical functions of coronavirus spike proteins, receptor recognition and membrane fusion must be considered. It is responsible for binding to the receptor on the host cell as well as mediating the fusion of host and viral membrane, including with a process driven by major conformational changes of the S protein. Zinc ion drug development is anticipated to be adopted by using ZAP Viral destruction via cell surface receptors and  $Zn^{2+}$  coordination pattern.

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## Bacteriolysis by Zinc Ions-Induced PGN Autolysins, Virucide by ZAP, ZBD, ZnONPs, and Prevailing Zinc Ions-Mediated HCoV's Matter

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