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Anti-Infective Activities for Bacterial Zn^{2+}-Induced Peptidoglycan Autolysins and Zinc-Binding Antiviral Proteins against Bacterial and Viral Infections

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Abstract

Anti-infective activities of bacterial Zn^{2+}-induced peptidoglycan (PGN) autolysins and zinc-finger antiviral proteins respectively are discussed against both bacterial and viral infections. Bacterial PGN autolysin AmiA for S. aureus amidase is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated molecule. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of Staphylococcus saprophyticus) is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats. AmiB catalyzes the degradation of PGN in bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell separation and PGN fragments release. 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 subpopulation of cells and complete flagellar synthesis. Thus, autolysin mediated bacteriolysis induced bacterial cell death can contribute to the bactericidal activities.

The other, 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, ZNFEB, including human protein variants, controls entry and exit from cell cycling in activated lymphocytes. The designed polydactyl zinc finger protein is prepared consisting HIV-1 type integrase fused to the synthetic zinc finger protein (ZNF) E2C. 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. ZNF Tsip1 controls Cucumber mosaic virus (CMV) RNA replication. Zinc-finger antiviral protein (ZAP) specifically inhibits the replication of certain viruses and promotes viral RNA degradation, ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism, and ZAP inhibit Sindbis virus translation may lead to the development of agents with broad activity against alphaviruses. ZAP also inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs and inhibits influenza A virus (IAV) protein that the short form of ZAPS inhibited IAV PB2 protein expression by reducing the encoding viral mRNA levels and repressing its translation. Thus, ZAPs findings provide insight into how antiviral components are regulated upon virus infection to inhibit virus spread. Viral infections spread based on the ability of viruses to overcome multiple barriers and move from cell to cell, tissue to tissue, and person to person and even across species with via two distinct routes. HIV-1 cell-to-cell transmission likely contributes to HIV-1 spread that the cell-to-cell transmission is sensitive to neutralization. The host cell restriction factors that mediate anti-influenza virus activity and viral countermeasures, limit influenza A infection that the potential to exploit restriction factors to limit disease caused by influenza and other respiratory viruses.

Keywords: *Bacterial PGN autolysin, Autolysin amidase, ZAP, Viral entry, replication and spread.*

Abbreviations

Aas=autolysin/adhesin of Staphylococcus saprophyticus, ABC=ATP-binding cassette, APC=antigen presenting cell, A. stephensi=Anopheles stephensi, B. abortus= Brucella abortus, B. subtilis= Bacillus subtilis, CBDs=cell wall binding domains, CBPs= choline binding proteins, C. difficile= Clostridium difficile, CKD=Chronic Kidney Disease, CMV= Cucumber mosaic virus, COPD=chronic obstructive pulmonary disease, 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=gelati-nase, HCV=hepatitis C virus, HD=hemodialysis, HIV-1=Human immunodeficiency virus type 1, IAV=influenza A virus, M. catarrhalis= Mora-xella catarrhalis, MCPs= Metallocarboxypeptidases, MDA= molondialdehyde, MIBRs= most probable immuno-protective B-cell epitope regions, MOV10= Moloney leukemia virus 10, MRB= multidrug bacteria, ORSs=oral rehydration solutions, ORT=oral rehydration therapy, P. aeruginosa= Pseudomonas aeruginosa, PBP2a=penicillinebinding protein2a, PGN=peptidoglycan, PGRPs=peptidoglycan recognition proteins, PSP=plasmid stabilization protein, 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 entoxin serotype B, SOD=superoxide dismutase, S. pneumoniae=Streptococcus pneumoniae, SSP=stable signal peptide, TB=pulmonary tuberculosis, TBVs= transmission-blocking vaccines, Tsip1=Tsi1-interacting protein 1, VRE=vancomycin-resistant Enterococcus faecium, ZAP=zinc-finger antviral protein, ZBD=zinc-binding domain, ZBL=zinc binding lipoprotein, ZNFs=Zinc-finger proteins, ZNFEB=EBV-induced zinc finger gene, ZnuA=Zinc uptake A.

Introduction

Zinc presence in respiratory, hematological disease, and endocrine systems has been of importance that 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 gastro-intestinal 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. Zinc influences apoptosis by acting on several molecular regulators of program-med cell death and zinc deficiency caused by malnutrition and foods with low bioavailability, aging, certain diseases, and deregulated homeostasis is a far more common risk to human health without intoxication [1]. Zinc is a key micronutrient that is present in all organs, tissues and body fluids that the dietary zinc deficiency produces reversible immune dysfunction, especially T-lymphocyte cell-mediated immunity, and is often virulent by intercurrent acute and chronic infections [2]. Zinc plays an important role in the chronic obstructive pulmonary disease (COPD) for pulmonary diseases and in serum zinc levels in children with acute respiratory infections. These important zinc effects have been reported including serum zinc levels for pulmonary tuber-culosis (TB) [3], Zn statuses [4], malondialdehyde (MDA) levels [5], macrophage Zn levels in efferocytosis [6], and a significant codeterminant Zn dyshomeostasis [7] for COPD patients. Zinc effects for respiratory infections are associated with ALRI case definition for acute lower respiratory infection of youg children [8], effect of zinc salts on respiratory syncytial virus replication [9], and a novel zinc binding essential for virulence (zev) against Haemophirus influenzae virus having been effectively on lung infection [10].

The other, the importance of Zn^{2+} ions in endocrine system is that its effect on growth, endocrine homeostasis, and thyroid function and on glucose metabolism that the deficiency of Zn^{2+} ions is associated with enhanced hepatic enzyme activity which catalyses the thyroid hormone inactivation [11]. Alterations in zinc-finger proteins (ZFPs) are involved in the development of several of diseases such as neurodegenaration, skin disease and diabetes[12]. The zinc plays sunidentified role where changes in zinc status over time may affect insulin activity that this unexplored concept would raise a whole new area of research into the pathophsiology of insulin resistance [13].

The role of zinc in cell death has apoptosis that the influence of zinc on apoptosis is tissue/cell type, zinc concentration, and expression of zinc transporters and zinc-binding proteins. Host zinc homeostasis changes in response to bacterial infections, including production of metal sequestering proteins and bombardment of bacteria with toxic level of zinc at

host-pathogen interface [14]. 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 [15]. 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 maximi-zed 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. 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 hemodia-lysis (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 [16].

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 [17]. 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 [18]. 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 [19].

Viruses are obligate intracellular 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 ondergo replication. The immune system has non-specific and specific mechanism that attack the virus in both phases of its life cycle which specific antibodies protect against viral infections and play an important role in antviral immunity, mainly during the early stage of the infection [20].

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 in flammatory response that intracellularly increased zinc can intoxicate engulfed pathogens and acts cytoprotective by promotion of neutralizing reactive oxygen species (ROS) and nitrogen species (RNS) [21].

In this review, enhancements of anti-infective activities of bacteriolysis by Zn^{2+} ions induced autolytic PGN activations and virucide by zinc-binding antivirus ptoteins and zinc-binding domains are discussed. Further, both the anti-bacterial and the anti-viral mechanisms have been clarified.

Zn^{2+} ions-induced PGN autolysin activation promotes anti-bacterial activity

Molecular structures of S. aureus and E. coli cell walls and 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 β-(1-4)-N-acetylmuramic acid (NAM) that are crosslinked by peptide stem chains attached to the NAM residues [22]. As shown in Fig. 1, 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 DD-endopeptidase. The other, for Escherichia coli (E. coli) cell wall as shown Fig. 2, there are endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals, and amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space [23]. The bacterial cell walls are a strong flexible mesh work of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodeled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to

maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase, glycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosy-lases as well as cell wall binding domains [24]. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial activities.

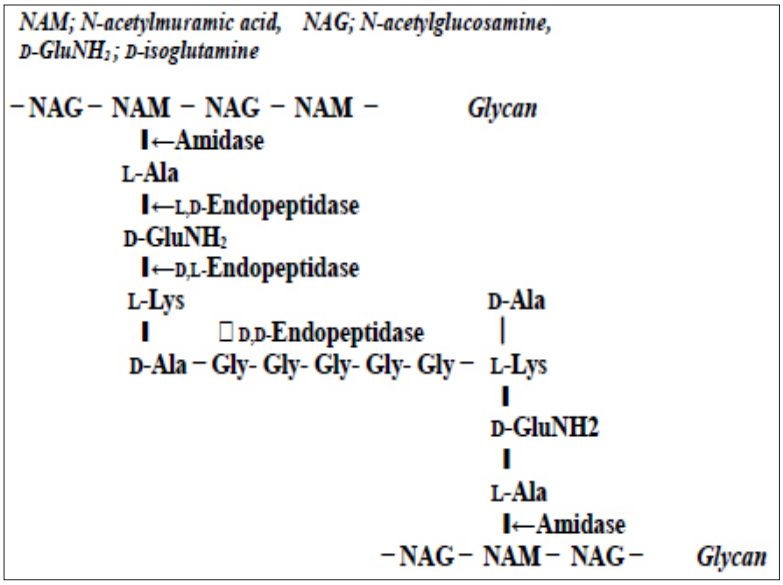

Fig 1. *Peptidoglycan structure and action sites of peptidoglycan autolysins against S. aureus PGN layer*

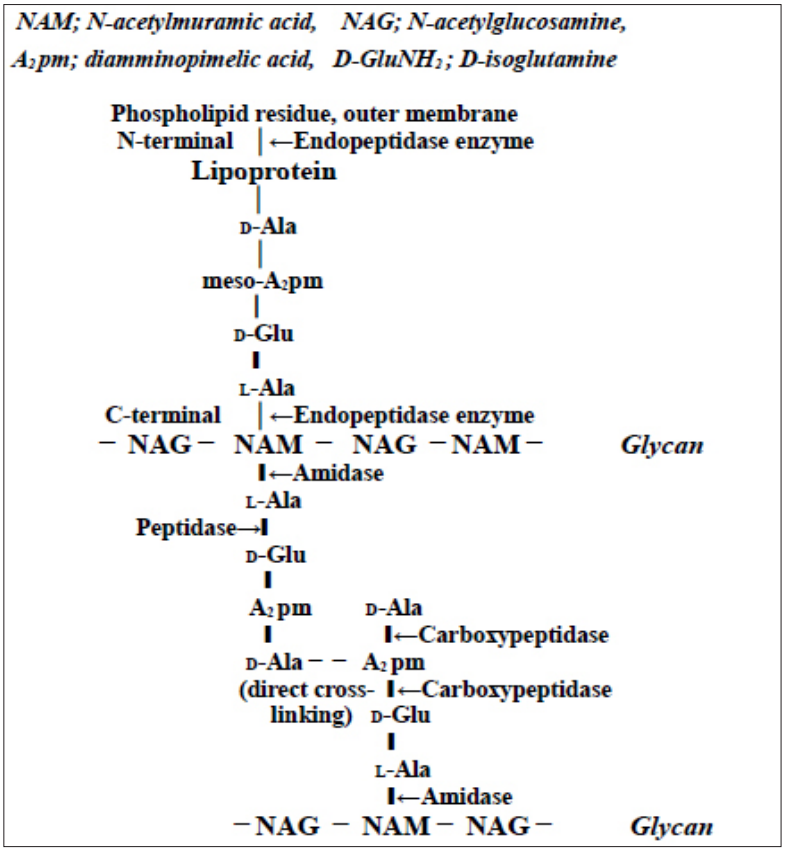

Fig 2. *Molecular structures of outer membrane lipoprotein and peptidoglycan layer in the E. colicell wall, and action sites of degrading enzyme of lipoprotein at C- and N-terminals and peptidoglycan autolysins*

Zn^{2+} Ions Induced Activated PGN Autolysins Promote Anti-Bacterial Activity against Gram-Positive Bacteria

S.aureus amidase AmiA is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated water molecule, in order to develop new therapeutics against MRSA [25].

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 [26]. 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 [27]. 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 [28]. 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 subsequ-ently, biofilms [29]. 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 [30]. 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 oef either lon A, and LytC, LytD, and LytF autolysins to population heterogeneity in B.subtilis [31]. 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 [32]. The biochemical and strucural staphylococcal Atl have successful cloaning, high level over-expression, and purification Atl proteins [33]. Major Atl autolysin also have an essential role in the early events of the fibronectin-binding proteins (FnBPs)-dependent S.aureus biofilm phenotype [34]. For the contribution of autolysins of PGN hydrolases to bacterial killing, there are N-acetylglucosaminidase (AtlA), two N-acetyl-muraminases (AtlB and AtlC) [35]. 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 subsequent cell separation can be modulated by a single GelE-mediated N-terminal cleavage event [36].

Zn^{2+} Ions Induced Degrading Enzyme of Outer Membrane Lipoprotein and PGN Autolysins Promote Anti-Bacterial Activity against Gram-Positive and Gram-Negative Bacteria

Amidase gene (AmiB) catalyzes the degradation of PGN in bacteria that the amiB gene was composed of 1,722 nucleotides and 573 amino acid which is involved in the separation of daughter cells after cell devision and inactivation of the amiB gene, resulting in a marked increases of sensitivity to oxidative stress and organic acids [37]. Amidase activity of amiC controls cell separation and PGN fragments release [38]. 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 [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 xopeptidases 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]. Metallocarboxypeptidases (MCPs) of the M32 family of peptidases exhibit a significant hydrolytic activity and different hydrolysis patterns against Trypanosoma brucei or cruzi [42]. Thus, zinc-depedent carboxypeptidase autolysin could adapt to be appreciable the anti-bacterial activities.

Table 1 represents anti-bacterial activities of bacteriolysis by Zn^{2+} ions induced activated PGN autolysins against Gram-positive thick PGN layer cell wall, and Gram-negative outer membrane lipoprotein and thin PGN layer cell wall.

Table 1. *Zinc induced anti-bacterial activities of bacteriolyses for Gram-positive thick PGN envelope cell wall, and Gram-negative lipoprotein and thin PGN layer cell wall*

Zn^{2+} Ions	*Gram-Positive* Thick PGN Layer Cell Wall
Zn^{2+}	**Zn^{2+} ions induced PGN autolysins** → Zn^{2+}, O_2^-, H_2O_2, ·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* ·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*

Zn^{2+} ions	*Gram-Negative* Cell Wall consisting of Outer Membrane Lipoprotein and Thin PGN Layer in Periplasmic Space	
	Outer Membrane Lipoprotein at C- and N-terminals	**Periplasmic Space Thin PGN Layer**
Zn^{2+}	→ Zn^{2+}, O_2^-, H_2O_2	→ Zn^{2+}, O_2^-, H_2O_2, OH^-, ·OH
	·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*	·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*

Anti-Viral Activities on Zinc-Induced Antiviral Immunity, Zinc-Finger Anti-viral Protein, Zinc-Binding Domain, and Membrane Fusion Protein

Zinc-Induced Antiviral Immunity and Inflammation

In order to maintain a healthy immune system, micronuritrient homeostasis is a key factor that the role of this micronutrient homeostasis during the course of infections and inflammatory response and how the immune system modulates zinc depending on different stimuli [43]. Zinc is an essential trace element that 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 [44]. 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 immuno-gens presenting the same antigenetic sites as the labile wild-type proteins efficiently elicit potently neutralizing antibodies [45].

Zinc-Finger Protein, Zinc-Finger Antiviral Protein, and Zinc-Induced Viral Spread Inhibition

Zinc-finger proteins (ZNFs) are one of the most abundant groups of proteins that have a wide range of molecular functions,in which ZNFs are able to interact with DNA, RNA, ubiquitin-mediated protein degradation, cell migration, and numerous other processes [12]. The novel EBV-induced zinc finger gene, ZNFEB, including human protein variants, controls entry and exit from cell cycling in activated lymphocytes [46]. The designed polydactyl zinc finger protein is prepared consisting HIV-1 type integrase fused to the synthetic zinc finger protein E2C that the integrase-E2C fusion proteins offer an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site [47]. Artificial zinc finger fusions 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 [48]. ZNF Tsip1 that the candidate genes encoded Tsi1-interacting protein 1 (Tsip1), ZNF Tsip1 strongly interacted with CMV 2a protein, controls Cucumber mosaic virus (CMV) RNA replication [49]. 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 [50].

Zinc-finger antiviral protein (ZAP) inhibits chiefly viral entry, replication, and spreading during viral infection. The ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation [51]. Expression of the ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism by which ZAP inhibit Sindbis virus translation may lead to the development of agents with broad activity against alphaviruses [52]. Moloney leukemia virus 10 (MOV10) protein consisting of a superfamily-1 RNA helicase, is a component of the RNA interference pathway, in which the MOV10 inhibits retrovirus replication that it could be actively involved in host defence [53]. ZAP inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs [54]. ZAP also inhibits influenza A virus (IAV) protein that the short form of ZAPS inhibited IAV PB2 protein expression by reducing the encoding viral mRNA levels and repressing its translation [55]. ZAP's stress granule localization due to cytoplasmic structure is correlated with antiviral activities that virus replication processes trigger stress granule formation and ZAP recruitment, in which these ZAPs findings provide insight into how antiviral components are regulated upon virus infection to inhibit virus spread [56].

Viral infections spread based on the ability of viruses to overcome multiple barriers and move from cell to cell, tissue to tissue, and person to person and even across species with via two distinct routes that either through the cell-free aqueous envilonment or , alternatively, by remaining cell associated and being passed on by direct cell-cell contact [57]. HIV-1 cell-to-cell transmission likely contributes to HIV-1 spread that the cell-to-cell transmission is sensitive to neutralization, but the effect of antibodies is often less marked than during cell-free infection [58]. ZAP-70 kinase regulates HIV cell-to-cell spread [59]. The host cell restriction factors that mediate anti-influenza virus activity and viral countermeasures,

limit influenza A infection that the potential to exploit restriction factors to limit disease caused by influenza and other respiratory viruses [60].

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 [61]. 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 [62].

Viral Membrane Fusion Protein And Phage Endolysin

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 [63]. 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 [63].

Bacteriophage (phage) is a virus that precisely infects bacterial hosts that after the completion of a replication inside the infected bacterial cell, newly formed phage particles need to be released outside the cell with the help of lytic enzymes, these lytic enzyme of endolysin, in which endolysins are bacteriophage-encoded peptidoglycan hydrolase [64].

Phage endolysin of cell wall binding domains (CBDs) is characte-rized in conjuction with their domain architecture, (non)necessity for the following lytic activity and a high/low specificity of their ligands as well [64].

Accordingly, anti-viral activities of zinc-finger antiviral proteins for virus entry, replication, and spreading are represented in Table 2.

Table 2. *Anti-viral activity of zinc-finger antiviral proteins for virus entry, replication, and spread*

Zn^{2+} ions	Anti-viral activity of Zn^{2+} in entry and replication	
	Adsorption/Entry	**Replication, DNA/RNA, Spread**
Zn^{2+}	→ Zn^{2+}, $\cdot O_2^-$, H_2O_2 ·EBV-induced zinc finger gene *ZNF*^{EB} controls entry and exit ·ZBD prevent viral entry and and GPC inhibit activate membrane fusion ·Zn-metalloprotease inhibits entry and cell-cell fusion	→ Zn^{2+}, $\cdot O_2^-$, H_2O_2, NO ·ZAP inhibits replication of MLV ·ZAP-mediated RNA degradation ·Zinc finger: virus decay ·Zinc finger proteinE2C; viral DNA specific sites ·Zinc finger protein Tsip1;Cucumber mosaic virus(CMV)RNA replication ·Artificial zinc finger fusion; HIV-1 tanscriptions ·ZAP-70 kinase regulates HIV cell-to-cell spread

Conclusions

Anti-infective activities of bacteriolyses by Zn^{2+} ions induced activated PGN autolysins and of virucides by zinc-binding viral fusion proteins are discussed, and the bacteriolytic and virucidal mechanisms are partially clarified.

Bacterial PGN autolysin AmiA for S.aureus amidase is acted on PGN binding and cleavage that amiA distinguishes PGN mostly by the peptide, and cleavage is facilitated by a zinc-activated molecule. The autolytic activity of the recombinant amidase of the Aas (autolysin/adhesin of Staphy-lococcus saprophyticus) is inhibited and is neccesary for the C-terminal GW repeats, not the N-terminal repeats. AmiB catalyzes the degradation of PGN in bacteria, resulting in a marked increases of sensitivity to oxidative stress and organic acids. Amidase activity of amiC controls cell separation and PGN fragments release. In these autolysins, zinc-dependent PGN autolysin of amidases may be enhanced and induced anti-bacterial 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 subpopulation of cells and complete flagellar synthesis.

Human peptidoglycan 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 bacterial cause of children's diarrea, in which antigen and antitoxin antibodies that neutralized both toxins that are associated with all cases of ETEC diarrhea. Thus, autolysin mediated bacteriolysis-induced bacterial cell death can contribute to the bactericidal activities. Bacterial autolysins enable the bacteriolyses of bacterial cell walls trim cell surface PGN to prevent detection by bacterial innate immune system. Autolysin mediated bacteriolysis- and zinc dependent lysis-induced bacterial cell death can contribute to the bactericidal vaccine activities, where PGN autolysins interact with biomolecules causing cell apoptosis leading to cell death.

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, in which the structure and mechanisms on viral membrane fusion protein are important problems. The novel EBV-induced zinc finger gene, ZNFEB, including human protein variants, controls entry and exit from cell cycling in activated lymphocytes. The designed polydactyl zinc finger protein (ZFN) is prepared consisting HIV-1 type integrase fused to the synthetic zinc finger protein E2C that the integrase-E2C fusion proteins offer an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site. ZFN Tsip1 controls Cucumber mosaic virus (CMV) RNA replication. ZFN ZCCHC3 binds RNA and facilitates viral RNA that ZCCHC3 is a co-receptor for the retinoic acid-inducible gene-1 (RIG-1) and MDA5 which is critical for RIG-1 like receptor (RLR)-mediated innate immune response to RNA virus.

Zinc-finger antiviral protein (ZAP) inhibits chiefly viral entry, replication, and spreading during viral infection. The ZAP specifically inhibits the replication of certain viruses and futhermore, an understanding becomes neccessary for ZAP-mediated viral RNA degradation. Expression of the ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism by which ZAP inhibit Sindbis virus translation may lead to the development of agents with broad activity against alphaviruses. Moloney leukemia virus 10 (MOV10) protein consisting of a superfamily-1 RNA helicase, is a component of the RNA interference pathway, in which the MOV10 inhibits retrovirus replication that it could be actively involved in host defence. ZAP inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs. ZAP also inhibits influenza A virus (IAV) protein that the short form of ZAPS inhibited IAV PB2 protein expression by reducing the encoding viral mRNA levels and repressing its translation. ZAP's stress granule localization due to cytoplasmic structure is correlated with antiviral activities that virus replication processes trigger stress granule formation and ZAP recruitment, in which these ZAPs findings provide insight into how antiviral components are regulated upon virus infection to inhibit virus spread.

Viral infections spread based on the ability of viruses to overcome multiple barriers and move from cell to cell, tissue to tissue, and person to person and even across species with via two distinct routes that either through the cell-free aqueous envilonment or , alternatively, by remaining cell associated and being passed on by direct cell-cell contact. HIV-1 cell-to-cell transmission likely contributes to HIV-1 spread that the cell-to-cell transmission is sensitive to neutralization, but the effect of antibodies is often less marked than during cell-free infection. The host cell restriction factors that mediate anti-influenza virus activity and viral counter-measures, limit influenza A infection that the potential to exploit restriction factors to limit disease caused by influenza and other respiratory viruses.

ZFN, ZBD, and membrane fusion protein specifically inhibit the entry and the replication of many viruses. The membrane fusion reaction, membrane interaction, conformational changes of specialized virus envelope proteins, and refolding reactions of specific fusion proteins have been discussed. Anti-viral activities of zinc-finger protein, zinc-binding domain, and membrane fusion protein are recognised by which highly diverse fusion proteins have converged on the same overall strategy to mediate a common pathway of membrane fusion, causing to lead enhancement of the anti-viral activity. Thus, an essential steps entry and replication of enveloped virus life cycle have been worthy of remark in fascination that these diverse viral fusion ptotein could be used in next-generation for therapeutic intervention in arenaviral disease.

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