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Review Article

Toxins and Their Role in Disease Fate and Treatment: An Overview on Bacterial Toxins' Functions

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Abstract
Some bacteria can produce toxins that give them an environmental advantage by modifying the life cycle of nearby competing cells. Generally, bacteria that produce toxins are more capable of causing disease compared to those that do not. Most bacterial toxins directly target key enzymes or molecules within cellular pathways of affected cells. Closer examination reveals that bacterial toxins are active molecules capable of crossing biological barriers or altering essential factors in living cells. This review discusses new applications of bacterial toxins in biotechnology and medicine. Recently, scientists have used toxins like botulinum toxin for cancer therapy and treating various disorders in humans and animals, including muscle problems and Blepharospasm. In this overview, we highlight the pathogenicity of bacterial toxins and their potential use in therapy and prevention. Although we focus on the most important toxins, many others are either harmful or beneficial for different purposes.

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Introduction

Some bacteria produce metabolites that apparently have adverse effects on target molecules; therefore, they are called bacterial toxins. Most of them have enzymatic activity with a structure mostly made of protein.[1] They can be experimentally produced through genetic engineering. The term toxin is intimidating, especially for cells lacking protection against it.[2] Toxins have a special structure or function that allows them to alter the molecules of target cells in harmful ways. However, some toxins can also have beneficial effects. These unique properties can be harnessed to solve problems or address deficiencies in various processes. Bacterial toxins often consist of separate domains, each with its own distinct function. These domains work together to achieve the toxin's ultimate goal. Some domains may target specific receptors on the target cell with high affinity or only affect certain substrates.[3] Other domains may possess activities or structures that are rare in natural or artificial molecules. The different components of bacterial toxins can be utilized directly or with structural modifications in various applications, ranging from pharmaceuticals and medicine to industrial uses.In this review, we explore several bacterial toxins that can be employed in medicine as therapeutic agents to treat diseases, sometimes serving as the primary cure.

Toxins as Pathogenic Agents

Food Poisoning

Certain exotoxins, endotoxins, and enterotoxins produced by specific bacteria can cause food poisoning, which is divided into two types. In the first type, at the time of ingestion, there are free bacterial toxins in the food without any living bacteria and in the other type, they are formed intracellularly after the bacteria invade the host.[4]

Staphylococcus aureus (S. aureus) is one of key organisms causing food poisoning with production of a wide type of enterotoxins (Staphylococcal enterotoxins (SEs)). Staphylococcal food poisoning (SFP) usually has a fast onset (2-8 h) and its symptoms include vomiting, nausea, and stomach cramping (with/without diarrhea).[5] These Symptoms usually do not need treatment and are eliminated after 1-2 days. The SEs

proteins are water-soluble, simple, heat- and pH-stable, with a molecular weight[6] between 28 to 35 kDa and, and are synthetized on the logarithmic phase or during the move from the exponential to the stationary phase of bacterial growth.[7] The quantities of SEs needed for active disease are between nanograms to micrograms. The five classical antigenic enterotoxin types A to E (SEA, SEB, SEC, SED, and SEE) are responsible for the SFP outbreaks.[5] All of these enterotoxins are superantigens, inducing an immune response with the generation of inflammatory cytokines. The Vβ elements of a T-cell receptor (TCR) directly bind to major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs) in order to subsequently stimulate massive cytokine release (IFN-γ, TNF-α, IL-2 and IL-6), and toxic shock inflammation.[8] Pervious study demonstrated that several important residues and major region responsible for contact between SEs with MHC II and TCR. For example SEA contain two important region with conserved amino acids to bind MHC II. The first region (Major binding site) is located near the amino terminus and contain H187, H225, and D227 residues. The F45 residue in second region responsible for minor binding site of SEA to MHC II. The high affinity between SEA and MHC II is likely to be achieved through cooperation between these two regions. The most common type of sudden outbreaks of SFP is SEA. The SEB is a primary cause of SFP after ingestion and the most dangerous form of SEs. In the U.S. Centers for Disease Control (CDC) list of warfare agents, SEB is considered as a category B agent. The SEC is the most frequent toxin isolated from bovine mastitis. The three antigenically distinct SEC subtypes with highly conserved proteins are SEC1, SEC2, and SEC3. The second most common serotype related with food poisoning is SED. DNA sequence identity indicates that SEA, SED and SEE are closely related and SEE has a high sequence homology (81%) with SEA.[9]

The animal model study for investigation of SEs emetic response especially SEA demonstrate that after adminis tration of SEA, migrates from the GI lumen to submucosa and binds to target submucosal mast cells and neurons. Binding of the SEA to it's an unidentified specific receptor on the surface of the submucosal mast cells cause the 5-HT to be released and binds to the 5-HT_3 receptor. This receptor abundantly expressed on enteric nerves, and this binding causes depolarization. Eventually, the vomiting reflex begins with depolarization of the vagus afferent nerves and stimulation of the brainstem vomiting site.[10,11] Another food poisoning illness with high fatality rate is botulism. It is caused by eating contaminated food with botulinum toxin, generated by the bacterium *Clostridium botulinum* under low-oxygen conditions. Botulinum toxin is divided into eight neurotoxin types (A, B, C [C1, C2], D, E, F, and G), which are serologically and antigenically different but structurally similar. Among these neurotoxins, types A, B, E and rarely F can cause disease in human and the other type is toxic in other animals.[12] The botulinum toxins are macromolecule with molecular weights between 300 kDa and 900 kDa, which is composed of the 150 kDa neurotoxin protein and one or more nontoxic proteins. The neurotoxin protein is cleaved by proteases into two subunit, heavy chain (approximately 100-kDa) and light chain (50-kDa), kept together by a disulfide bond. The botulinum toxin type A is stronger with long-term symptoms than other types in human. The most common symptoms of type A include flaccid paralysis, headaches, myofascial pain syndrome, and focal dystonia. The botulinum toxin type B (1290 amino acids long) consists heavy chain with 850 amino acids and light chain with 440 amino acids. After translocation into the eukaryotic host cytosol, the light chain blocks neurotrans mitter release and heavy chain is responsible for host epithelial cell transcytosis. The botulinum toxin type E has 1250 amino acids (829 amino acids for heavy chain and 421 amino acids for light chain has). After translocation into the eukaryotic host cytosol, the light chain of type E inhibits neurotransmitter release by acting as a zinc endopeptidase.[13]

Biological Warfare

Like chemical substances, bacterial toxins can be used as warfare agents. The US Army Medical Research Institute of Infectious Diseases (USAMRIID) expresses that botulinum toxin and staphylococcal enterotoxin B can be used as weapons.[14] In addition, based on the US Centers for Disease Control and Prevention (CDC), *Clostridium perfringens* (ETX)-derived epsilon toxin is considered as a bioterrorism agent. From these bacterial toxins, just botulinum toxin is in category A and it has attracted the attention of terrorist groups. SEB and ETX are accounted as Category B. The agents of Category A such as botulinum toxin are much more dangerous than category B, because: 1- they can be easily disseminated, 2- have high mortality rates, and 3- might cause public panic.[15]

The specifications and properties of the botulinum toxin and staphylococcal enterotoxin type B were explained in the "food poisoning" section. ETX belongs to the pore-forming family of toxins and is generated by *Clostridium perfringens* types B and D. *Clostridium perfringens* is one of the most important anaerobic pathogens of humans and domestic animals and based on the production of one or more toxins, falls into into five types (A to E). *Clostridium perfringens* is a spore-forming, rod-shaped, and anaerobic Gram-positive bacterium, which produces about 20 toxins. The ETx causes enterotoxaemia, dysentery and enteritis in the domestic ruminants, mainly sheep.[16]

The molecular weight of ETX is about 33 kDa. First, it's produced as protoxin with poor toxicity. For the activation, protoxin should be enzymatically cleaved by host α-

chymotrypsin, trypsin or λ-protease that is encoded by *C. perfringens* and about 45 amino acids in mature protein are removed from N- and C-terminal peptides. The molecular weight of mature protein (46-328) form is 28.6 kDa. The toxicity of active form of ETX increases by approximately 1000-fold in contrast to the prototoxin and this form of the toxin induces perivascular edema in various tissues (especially kidneys and brain) and causes central nervous system (CNS) disease after passing through the blood–brain barrier.[17] Epsilon toxin contain 3 domain (D1, D2 and D3). Domain 1 contain an α-helix and a three-stranded anti-parallel sheet. Tyr42, Tyr43, Tyr49, Tyr209 and Phe212 residues in D1of etx are responsible for receptor binding. Domain 2 in epsilon toxin is a β-sandwich structure with a five-stranded sheet and a β-hairpin that involved in β-pore-forming of toxins. The D3 is a β-sandwich containing a one four-stranded sheet and one three-stranded sheet. It is contain the cleavage site for toxin activation.[18,19] The pervious biochemistry and mutagenesis study demonstrate that His106 and His 149 residues are required for the active site, and Trp and Tyr residues are necessary for the binding to target cells . The Etx mode of action begins with binding of activated form of toxin to its protein receptor. Subsequent on the membrane surface the oligomerizes a heptameric prepore and finally prepore insert to the surface and complete pore formation.[20]

Cancer-Inducing Agents

Cancer development is a multifactorial process, characterized mainly by genetic mutations and epigenetic alterations.[21] Nowadays, the relationship between bacteria-produced toxins and cancer development is emerging. The relationship was first discovered between *Helicobacter pylori* and gastric cancer by IARC Working Group in 1994.

Toxin-mediated carcinogenesis can occur following direct DNA damage[22,23] or alterations of multiple cellular processes such as proliferation, differentiation, response to stress, and apoptosis; resulting in cellular aberrations or cell cycle arrest.[24,25] DNA damages like single strand breaks (SSBs) and double strands breaks (DSBs), if keep unrepaired or erroneously repaired, can lead to structural rearrangements and genomic mutations associated with carcinogenesis and other diseases.[26-28] For example, colibactin and cytolethal distending toxin (CDT) are bacterial genotoxins inducing DNA damages.[29] CDT is produced by several Gram-negative human mucosal pathogens, including *S. typhi*, *Escherichia coli,*[30] *Shigella dysenteriae*, *Campylobacter jejuni,*[31] *Aggregatibacter actinomycetemcomitans,*[32] *Haemophilus ducreyi*, *Helicobacter sp,*[33] *typhoidal*, and some non-typhoidal *Salmonella* serovars.[34,35] The toxin is consisted of 3 subunits, CdtA, CdtB, and CdtC. A single operon encodes the CdtA and CdtC, which are necessary for the transfer of CdtB to host cells.[36] The CdtB is an enzymatically active subunit similar to DNase I, which can induce DSBs or SSBs, cell cycle arrest in G2/M, and eventually apoptotic death.[37] For example, it has been reported that CdtB of *Helicobacter hepatics* can increase intestinal carcinogenesis in 129Rag2-deficient mice by enhancing DSBs and activation of the TNFα-initiated IL-6-STAT3 signaling pathway.[38] Notably, CdtB has another enzymatic activity called phos phatidylinositol-3,4,5-triphosphate (PIP3) phosphatase that can stimulate cell cycle suppression through disrupting the phosphoinositide 3-kinase signaling pathway (Figure 1).[39,40]

Colibactin is a potent toxin generated by strains of the B2 phylogenetic group of *Escherichia coli*, containing a 54-kb polyketide synthetase (pks) island.[41] The genotoxin induces DSBs in host cells by blocking the mutL homologue 1 (MLH1) mismatch repair protein, which results in chromosome aberrations and cell cycle arrest in the G2/M phase.[42,43] Moreover, it has been shown that colibactin-producing *E. coli* can induce the expression of microRNA-20a-5p, which promotes the invasive and metastatic abilities by suppressing small ubiquitin-like modifier 4 (Smad4) expression in colorectal cancer cells (Figure 1).[44-46] Several bacterial toxins have been reported to drive carcinogenesis by interfering with cellular signalling mechanisms related to cell proliferation. For example, i toxins such as CNF1, VacA, and CagA, and BFT that are produced by *Escherichia coli, Helicobacter pylori*, and enterotoxigenic *Bacteroides fragilis*, respectively. A 110-kDa protein toxin called cytotoxic necrotizing factor 1 (CNF1) is generated by a pathogenic *E. coli* strain that has roles in the activation of the Rho, Rac, and Cdc42 GTPases, three subfamilies of the Rho GTPases family, *in* eukaryotic cells. Activation of the subfamilies occurs by deamidation of a glutamine residue for GTP hydrolysis and leads to a number of different phenomena associated with colon cancer, including multi nucleation , activation of NF-κB,[47] inflammatory cytokines and, re-entering the cell cycle (Figure 1).[48]

Most *H. pylori* strains containing specific toxins such as vacuolating toxin (VacA) and cytotoxin-associated gene A (CagA) are related with enhanced gastric cancer risk.[49] VacA is a pore-forming secreted toxin regulated by secretion system subunit protein A (SecA). SecA hydrolyzes ATP to drive VacA out of the bacterial cell membrane; upon translocation to the gastric epithelial cells,[50,51] VacA leads several alterations in these cells, including mitochondrial perturbations, disruption of endosomal/lysosomal function, membrane channel formation, apoptosis, vacuolation, plasma membrane potential depolarization, and diffusion of various ions like chloride, bicarbonate, and urea, and activation of mitogen protein kinase (MPK).[52,53] Additionally, VacA can induce autophagy in gastric epithelial cells by endoplasmic reticulum (ER) stress through increasing the expression of tribble pseudokinase 3 (TRIB3), which plays an important role in induced autophagy by ER stress.[54] Therefore,

knockdown of the effector proteins related to ER stress can remarkably reduce VacA-induced cell death.[54] Importantly, VacA can promote gastric tumorigenesis by the induction of the Wnt/β-catenin signaling and activating CCND1 (Figure 1).[55]

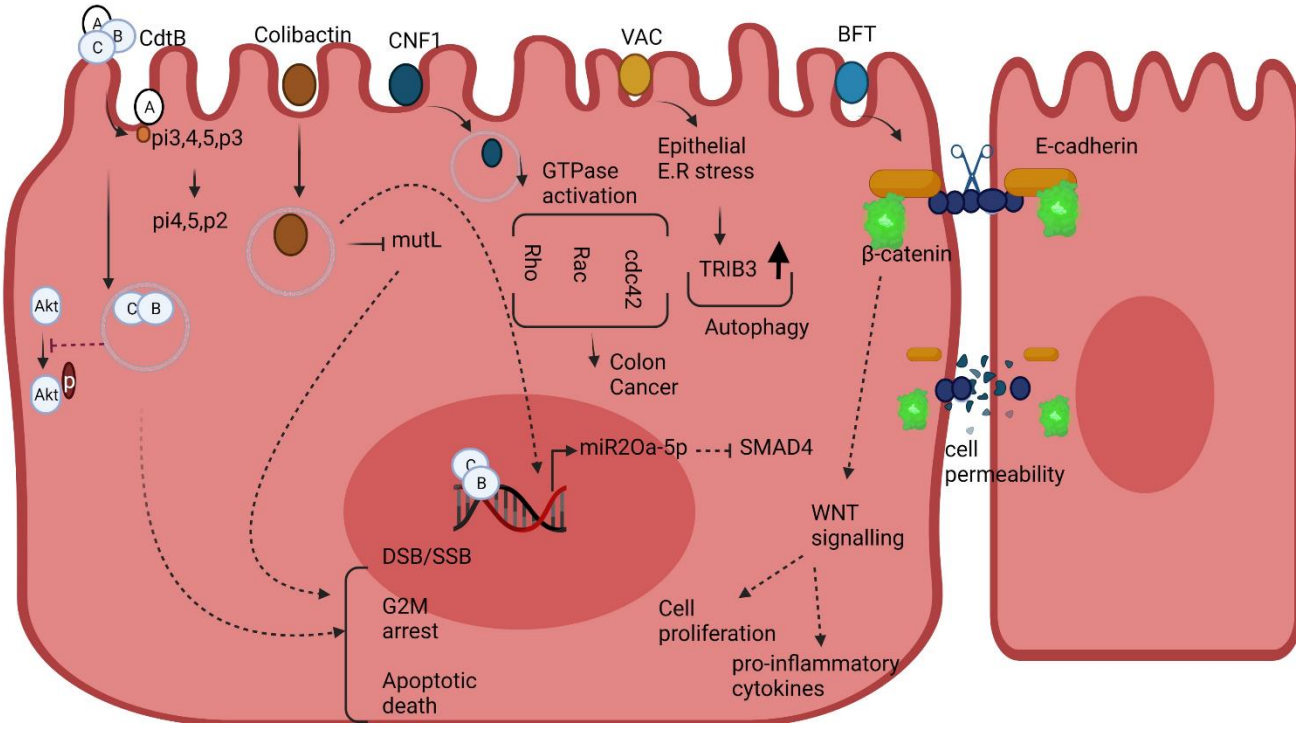

Figure 1. Different Toxins Involed in Cancer Inducing Process. CdtB enter the cells and with DNase I like function starts IL-6-STAT3 signaling pathway and cell cycle suppression. Colibactin by blocking the MLH1 that it is responsible to the mismatch repair protein, initiates chromosome aberrations and cell cycle arrest in the G2/M phase. CNF1 with the activation of the Rho, Rac, and Cdc42 GTPases leads to a number of different phenomena associated with colon cancer, including multinucleation, activation of NF-κB, inflammatory cytokines and, re-entering the cell cycle. VacA can induce autophagy in gastric epithelial cells by endoplasmic reticulum (ER) stress through increasing the expression of tribble pseudokinase 3 (TRIB3), which plays an important role in induced autophagy by ER stress. BFT after binding to the colonic intestinal epithelial cells cleaves the extracellular domain of E-cadherin. After that, mediated by intracellular degradation with some unknown protease, the activation of β-catenin/Wnt and NF- κB signaling pathways occur.

CagA harbors in Cag pathogenicity island (cagPAI). The cagPAI encodes a type 4 secretion system helping the injection of CagA when *H. pylori* binds to the target cell.[56] After internalization, CagA is localized to the inner side of the plasma membrane and becomes rapidly tyrosine phosphorylated by the kinase family of Src (SFKs),[14] Then, interaction of the phosphorylated CagA with host molecules leads to morpho;ogical chnges such as cell elongation.[57] Basically, formation of cell elongation needs actin polymerization through a complex called Arp2/3 and this process is usually controlled by the members of the WASp/SCAR/WAVE family of scaffold proteins.[58,59] The WASp and N-WASp directly activated by Cdc42 and also Scar/WAVE family indirectly activated by Rac. It is while a 2007 study showed that CagA-mediated cell elongation was as a cell retraction defect independent of noted proteins.[60] Additionally, CagA can cause a series of phenomena like c-Jun N-terminal kinase (JNK) signaling, that are linked to progression of cancer[61] and induce nuclear β-catenin accumulation, affecting transcriptional regulation of Wnt-target genes and increasing cell proliferation (Figure 1).[55,62]

A 20- kDa zinc metalloprotease called *B. fragilis* toxin (BFT) is produced by enterotoxigenic *B. fragilis*. This metalloprotease is associated with various disease, such as acute diarrheal, inflammatory bowel disease and colorectal disease (CRC).[63] After binding of BFT to colonic intestinal epithelial cells, the extracellular domain of E-cadherin is cleaved.[64] Then, intracellular degradation occurs with some unknown protease(s), followed by the activation of β-catenin/Wnt and NF- κB signaling pathways. This leads to the enhanced cell proliferation and the production of pro-inflammatory cytokines (Figure 1).[63,65]

Cardiovascular Diseases

Atherosclerosis-related diseases such as chronic heart disease (CHD), are the most common type of cardiovascular diseases (CVDs). CHD can occur due to narrowing of the coronary arteries by a buildup plaque, which is made up of lipid, calcium, macrophage, and other substances found in the blood. Since these plaques are often unstable, they eventually rupture to form a thrombus (blood clot) that can lead to restriction or even blockage of the flow of oxygen-

enriched blood to the heart and other parts of the body.[55,56] Hypertension,[57] high cholesterol,[58] smoking,[59] diabetes,[57] vitamin D deficiency,[60] and obesity[61] are known as major risk factors for developing the atherosclerosis-related diseases (Figure 2). Additionally, some bacterial toxins can also be considered as a risk factor for these diseases due to the induction of uncontrolled secretion of pro-inflammatory cytokines and impaired endothelium permeability.[62-65]

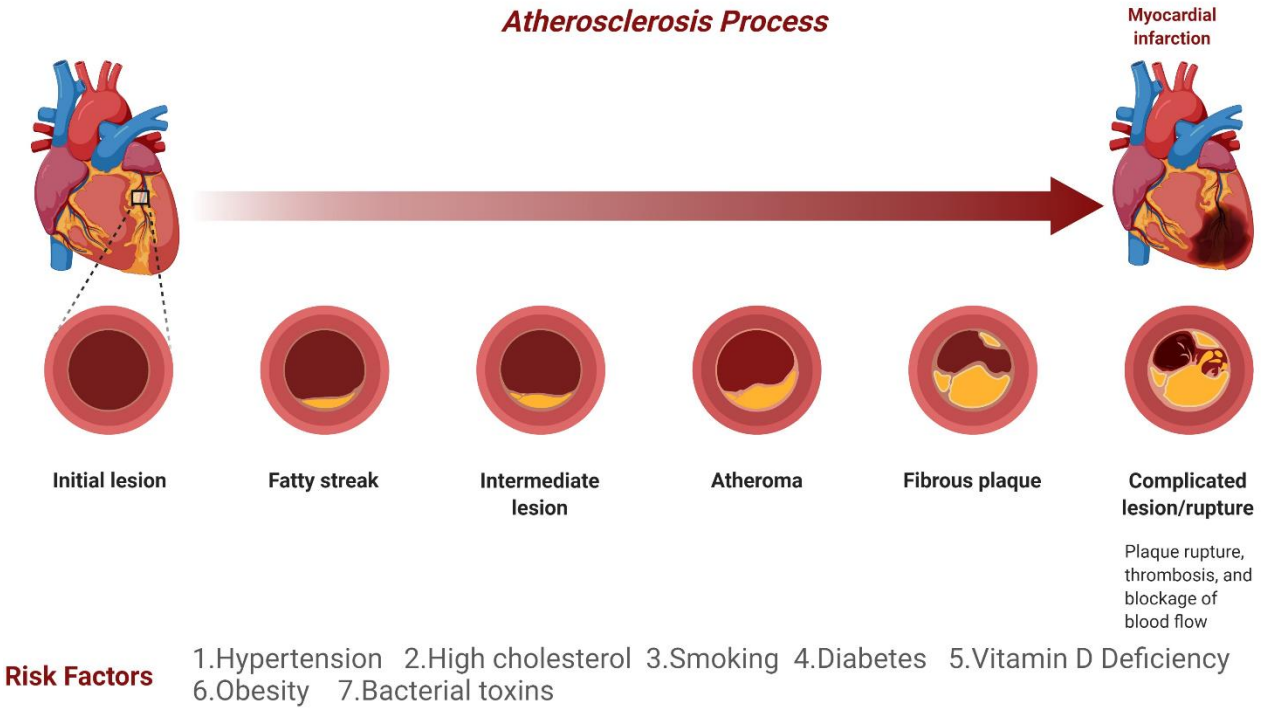

Figure 2. Chronic heart disease is caused by the accumulation of plaque in the arteries that carry blood to the heart and other organs. Plaque is composed of cholesterol and other substances that have accumulated in the artery. Which may cause a partial or complete blockage of blood flow; there are many significant risk factors for acquiring this condition.

The effect of *H. pylori* infection on the progression of CHD was first reported by Mendel *et al* in 1994, following evaluation of the *H. pylori* IgG and IgA levels in patients with CHD.[66] In spite of such a questionable observation, recent publications indicate that patients infected with highly virulent *H. pylori* strains containing CagA are more likely to develop CHD. This relation is probably due to the stimulation of pro-inflammatory responses, resulting in enhanced levels of cytokines such as IFN-γ, IL-1β, IL-6, IL-8, TNF-α, coagulation factors especially factor VII, fibrinogen, and thrombin.[63,67-69]

The largest family of toxins are the pore forming toxins (PFTs), generated by several pathogenic bacteria, including, *Staphylococcus aureus,*[70] *Streptococcus pneumoniae,*[71] group A and B streptococci,[72,73] *Listeria monocytogenes,*[74] *Clostridium perfringens,*[75] *Bacillus subtilis,*[76] *Escherichia coli,*[77] and *Mycobacterium tuberculosis.*[78] PFTs can rapidly induce macrophages to secrete the IL-1β and HMGB-1 in a NLRP3 inflammasome and cathepsin B-dependent manner.[79,80] High-mobility group box 1 (HMGB1) is a novel cytokine, a DNA-binding protein, which stimulates monocytes to secrete pro-inflammatory cytokines and is highly expressed in atherosclerotic lesions. Several recent studies indicate that HMGB1 can have an important role in regulating the progression of atherosclerosis.[81-83]

Leukotoxin (LtxA) is a 113-kDa protein from RTX toxin family, produced by *Aggregatibacter actinomycetemcomitans.*[84] Recent studies indicate that LtxA, like lipopolysaccharides (LPS), can play a role in the formation of atherosclerosis by increasing endothelial permeability, followed by the induction of endothelial apoptosis and increasing endothelial adhesion molecules.[64,85]

Toxin-Antitoxin and their Role in Bacterial Pathogenesis

Toxin-antitoxin (TA) systems initially were seen on Plasmid and were later found on chromosomes as well. Typically found in operons, TA systems consist of a stable toxin and a less stable antitoxin, which can be either a protein or RNA. The toxin interferes with cellular processes, causing growth arrest or cell death, while the antitoxin counteracts the toxin's effects. Toxins play a role in regulating various biological functions, such as plasmid maintenance through post-segregational killing (PSK), where toxin-induced cell lysis eliminates plasmid-free cells. This dependency on plasmids earned plasmid-encoded TA systems the name of

plasmid addiction modules. Bacterial chromosomal TA systems are implicated in activities like stress response, biofilm formation, phage inhibition, virulence, and persistence, showcasing their functional versatility under different stress conditions. Nonetheless, the exact contributions of TA systems to stress response, biofilm formation, and antibiotic persistence creation remain topics of debate. These systems are prevalent in pathogenic organisms and have been linked to bacterial pathogenesis in multiple studies, demonstrating their importance in infection establishment, intracellular survival, adaptation to host defenses, and long-term infection. This review zooms in on the distinct characteristics of each TA system type and recent research exploring their roles in bacterial pathogenesis.[66]

Numerous research studies have shown that the TA system plays a role in bacterial pathogenesis and virulence through infection models. So far, type I, II, and VII TA systems have been shown to have an impact on bacterial virulence. The subsequent sections will explore examples of how TA systems contribute to bacterial virulence in Table 1.

Table 1. TA Systems in Bacterial Pathogenesis

Pathogen	Infection model	Mechanism of pathogenesis	TA system		Role of TA system	Ref
Staphylococcus aureus	*Fitness,Intracellular survival*	*Host cell lysis*	*Type I TA system*	*sprG1-sprF1*	*Hemolysis*	*(67)*
Enterococcus faecalis	*larvae Galleria mellonella, macrophages and mice*	*Hypervirulence, Intracellular survival, colonization*		*ef0409-ef0408*	*Adaptation to host stresses, regulation of expression of metabolic enzymes*	*(68)*
Salmonella typhimurium	*Fibroblast*	*Fitness,Intracellular survival*		*tisB-istR, ldrArdlD and hok-sok*	*Regulation of growth of bacteria inside the host*	*(69)*
Extraintestinal pathogenic Escherichia coli (ExPEC)	*Mice*	*Colonization*	*Type II TA system*	*yoeB-yefM*	*Regulation of growth of bacteria inside the host*	*(70)*
ExPEC	*Mice*	*Fitness, Intracellular survival*		*pasT-pasI*	*Adaptation to host stresses, Tolerance to antibiotics, Biofilm and persister formation*	*(70)*
Haemophilus influenzae Haemophilus influenzae	*Epithelial tissue*	*Survival inside the host, Colonization, Persistent infection*		*vapC-vapB and vapDvapX*	*Persister formation*	*(71)*
S. typhimurium	*Macrophage, Fibroblast, and Epithelial cell*	*Fitness, Intracellular survival*		*vapB2-vapC2 and T4-A4*	*Regulation of growth of bacteria inside the host, Metabolic readjustment*	*(69)*
S. aureus	*-*	*Regulation of virulence factors*		*mazF-mazE*	*Regulation of growth of bacteria inside the host, Metabolic readjustment*	*(72)*
Mycobacterium tuberculosis	*Guinea pig*	*Regulation of virulence factors*			*Regulation of growth of bacteria inside the host, Metabolic readjustment*	*(73)*
M. tuberculosis	*Guinea pig*	*Infection, Fitness*		*higB1-higA1*	*Regulation of expression of the genes involved in virulence, detoxification, and adaptation*	*(74)*
Neisseria gonorrhoeae	*Epithelial cell*	*Intracellular survival*		*fitB-fitA*	*Regulates rate of intracellular replication and growth of bacteria*	*(75)*
Vibrio cholerae	*Mice*	*Intracellular survival colonization*		*relE-relB*	*Biofilm and persister cell formation*	
ExPEC	*Mice*	*Colonization*	*Type VII TA system*	*hha-ybaJ*	*Regulation of growth of bacteria inside the host*	*(70)*
M. tuberculosis	*Guinea pig*	*Colonization*		*menT2-menA2*	*Regulation of growth of bacteria inside the host*	*(76)*

Toxins as Healer Agents

Cancer Treatment

Several toxins-derived bacterial pathogenesis factors have activity as virulence factors. Some of these toxins possess the ability of entering the mammalian cells and changing their substrates in the cytosol.[77] The most important toxins in this category include diphtheria toxin, shiga toxin and *Pseudomonas aeruginosa* exotoxin A (PE). Such toxins have been frequently engineered as the cell killing component of the immunotoxins. Immunotoxins are the fusion proteins contain an antibody, its parts or a ligand fused with catalytic domain of a toxin.[78-83] So far, two immunotoxins have been approved by Food and Drug Administration (FDA). FDA approved the Denileukin Diftitox (Ontak) as the first approved immunotoxin applied against the cutaneous T-cell lymphoma (CTCL). The Ontak has been formed with linkage of the human interleukin-2 (IL-2) and fragment A of diphtheria toxin (translocation and catalytic domain). Despite all its successes, it has severe side effects including disruption of color vision or blurred vision, diarrhea, nausea,

muscle pain, skin rash, and flu-like symptoms. Another side effect called vascular leak syndrome (VLS) that is more important than the others, is caused by its off-target toxicity.[84] For this reasons, Ontak was approved by FDA in 2002 and got a black box warning label by FDA in 2006.[77] In September 2018, the second immunotoxin called Moxetumomab Pasudotox (Moxe) against hairy cell leukemia (HCL) was approved by the FDA.[85] Moxe was genetically engineered as a single chimeric molecule consisting of a fragment of *Pseudomonas* exotoxin A with 38-kDa component linked to a single chain fragment variable (scFV) antibody against the CD22 marker on B-cells. The results of Moxe in an open-label trial with 80 patients showed 75% objective response rates, while intense side effects were reported only in less than 5% of patients as hemolytic uremic syndrome, hypertension, and febrile neutropenia. Nevertheless, both immunotoxins have been used for the treatment of non-solid tumors, while the most important challenge for using immunotoxins, especially in the treatment of solid tumors, is immunogenicity and VLS syndrome. Our team designed several PE38-based immunotoxins against different target. We produced and characterized PE38-P4A8 immunotoxin consisting of scfv that targets fibroblast growth factor-inducible 14 (Fn14) and *Pseudomonas* exotoxin 38 (PE38). The PE38-P4A8 had remarkable cytotoxic effects on HT-29 and A549 cell lines.[86] The 1F12-PE38KDEL is the first scfv-based immunotoxin recognizing the ephrin type-A receptor 2 (EPHA2).[87] This immunotoxin in a nanomolar range, was significantly able to bind and kill the breast cancer lines with high EPHA2 on cell surface.[88]

Some of bacterial exotoxins are able to disrupt both plasma membrane and extracellular matrix of animal cells by pore formation or enzymatic hydrolysis. These include α-toxin (from *Clostridium perfringens*) with phospholipase C activity, streptokinase (from *Streptococcus pyogenes*) with plasminogen to plasmin hydrolysis, and collagenases (from clostridia). Other toxins with pore-forming ability include streptolysin O from *S. pyogenes*, listeriolysin O from *L. monocytogenes*, α-toxin from *S. aureus*, hemolysin from *E. coli*, perfringolysin O from *C. perfringens*, aerolysin from *A. hydrophila*, and pneumolysin from *Streptococcus pneumonia*.

A diverse set of toxins are super antigens produced via staphylococcal and streptococcal bacterial strains and can stimulate high inflammatory responses and induce acute toxic shock.[89] The super antigens family mostly is structurally diverse. The staphylococcal enterotoxin B[14] and toxic shock syndrome toxin-1 (TSST-1) are two members of super antigens family playing role both in human illnesses and cancer treatment. Currently, applying the SEB-exosome combination against pancreatic cancer has shown high ability in the apoptosis induction.[90] We also showed that the TGFαL3-SEB chimeric protein can induce apoptosis through activation of caspases 3 and 9.[80] Of note, Izquierdo et al. have revealed that the SEB-induced apoptosis can be reduced by inhibition of caspase activation pathways. Increase in the activity of the cytotoxic T lymphocyte and also in cytokine production happens after using staphylococcal enterotoxin A super antigen (SEA) as a potent inducer of T cells. That's due to the binding of SEA to major histo compatibility complex class II molecules from the T cells containing T-cell receptor beta chain domains. Our investigation showed the lymphocyte cells exposed to the recombinant SEA can secretes large amounts of interferon γ (INF- γ) and tumor necrosis factor (TNF).[91]

Application in Neurological Disorders

Botulinum toxin with seven serotypes (A–G) is formed by a heavy and a light chain fused with a disulfide bond.[92] Heavy chain can be internalized to the cell by binding to the presynaptic cholinergic nerve terminals. Light chain has toxic effects and acts as a zinc-endopeptidase and cleaves the target proteins within the cytosol (Figure 1). Botulinum toxin can disrupt the neuromuscular transmission at neuromuscular junction, resulting in denervated muscle. It is able to reduce salivation and sweating with activity in other peripheral cholinergic synapses. FDA approved the BoNT/A and BoNT/B for applying in cervical dystonia[93] on December 2000. The key electrophysiological effect of BoNT is a decrease in the intensity of compound muscle action potentials (CMAPs) after single or repeated supramaximal nerve stimulation with 3-month duration. CD or spasmodic torticollis is a type of cervical musculature. The spinning, tilting, flexion, or expansion displacements of the head are caused by muscle spasms in the neck. Oral drugs like anticholinergic agents, baclofen, and benzo diazepines often have many side effects. Botulinum toxin therapy is the most common treatment for CD, providing relief to up to 85% of patients. It has been shown that BoNT can improve head posture, discomfort, and range of movements (Figure 3).

Vaccination

Toxoid or toxin product-based vaccinations are pivotal preventative actions against pathogenic agents, and developments of recombinant-based technologies, propose a type of different effective vaccines for humans and animals.[94] The toxoid vaccines have been obtained against diphtheria and tetanus and these vaccines have been available alone or in different combinations.[95] There are diphtheria–tetanus–pertussis (DTP) vaccines for childhood immunization programs worldwide. So, developments of these vaccines are quite mature. Among them, only pertussis is in acellular form. Also, other DTP combination vaccines are developed such as DTP-HBV–Hib vaccine (phase not expressed),[95] DTwP–HBV–Hib (phase not expressed) and DTwP–HBV (phase III in processing) vaccines.[95] In recent years, recombinant botulinum vaccines have had significant

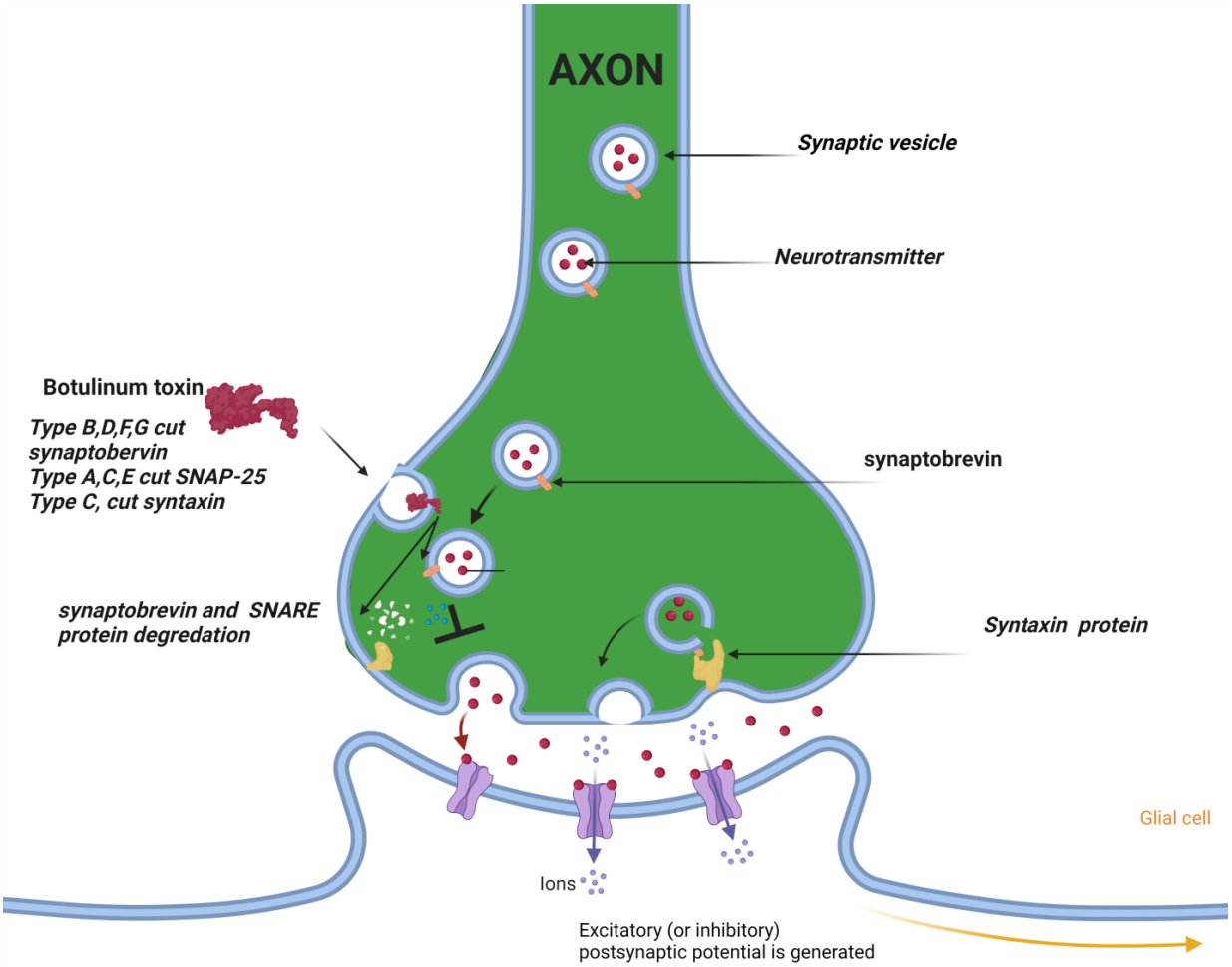

Figure 3. Botulinum toxin may attach to the synaptic membranes internalized into the synaptic terminal. The Type B, D, F, G of Botulinum toxin cut synaptobervin, Type A, C, E cut SNAP-25 Type C, cut syntaxin, as a result, plasma membrane-fusing acetylcholine vesicles cannot fuse, and its exocytose is blocked.

progress. The utilization of the non-toxic binding domain part of the BoNT heavy chain (HC) has appeared to be a potential replacement to toxoid-based vaccines. In comparison with the traditional toxoid vaccine, higher antibodies rate with few adverse reactions were shown in vaccinated horses.[96] Also, the multi-valent vaccines containing BoNT/A-HC, BoNT/B-HC, and BoNT/E-HC have been able to protect immunized mice in a challenge with all of toxins.[97] Now, some researchers are interested in creating subunit vaccines by the binding domain of the tetanus toxin heavy chain (TeNT-Hc), and in this regard, good results were obtained in rats and mice but required additional vaccinating dosages in monkeys.[98] We separately produced the TeNT-Hc, BoNT/A-HC, and entrotoxigenic *Eshirishia coli* heat labile toxin B subunit (LTB) in *Escherichia coli* BL21 DE3 host containing pET28a vectors. In this study, after immunization of mice, the frequency of specific memory B-cells were measured after 3 and 6 months post-immunization and a significant correlation was detected between frequency of total memory B-cells and duration of humoral immunity.[87]

C. perfringens strains fall into seven toxinotypes (A–G) depending on production of toxin: *C. perfringens* enterotoxin (CPE), iota (ITX), alpha (CPA), beta (CPB), epsilon (ETX), or the necrotic enteritis beta-like toxin (NetB).[99] The toxin types A (CPA), C (CPA and CPB), and F (CPA and CPE) are able to affect humans and other toxinotypes only cause disease in animals.[100] The multi-valent combination of ETX, CPA, and CPB, toxoids or bacterin-toxoid(s) are available in the market as veterinary vaccines against *C. perfringens*.[101] However, there are no commercial toxoids that give protection against other *C. perfringens* toxins including ITX, CPE, or NetB. Also, multiple subunit vaccines are developed against CPE, CPA, NetB, and ETX that confer promising protective responses in several animals.[102, 103]

Given that the anthrax toxin is in the top of bioterrorist list agents, production of neutralizing vaccine and antibody against this horrible toxin has particular importance. Several protective antigen (PA)-based vaccines are developing against anthrax toxin. PA (the non-toxic fragment responsible for cell-binding and transporting other fragments into the cell) along with edema factor (EF), and lethal factor (LF) are three fragments encoded by plasmid pXO1 and contribute to

B. anthracis pathogenicity. Epicutaneous anthrax vaccine and intranasal anthrax vaccines are created by PA and germination-associated anthrax antigens, and one of them is in the pre-clinical testing.[104] rPA102 and Thraxine™ are composed with only recombinant PA and are going through phase II clinical trials.[105] Medarex is developing the specific human monoclonal antibody against the mannose receptors as a delivery vehicle for anthrax PA by as targeted anthrax vaccine.[106]

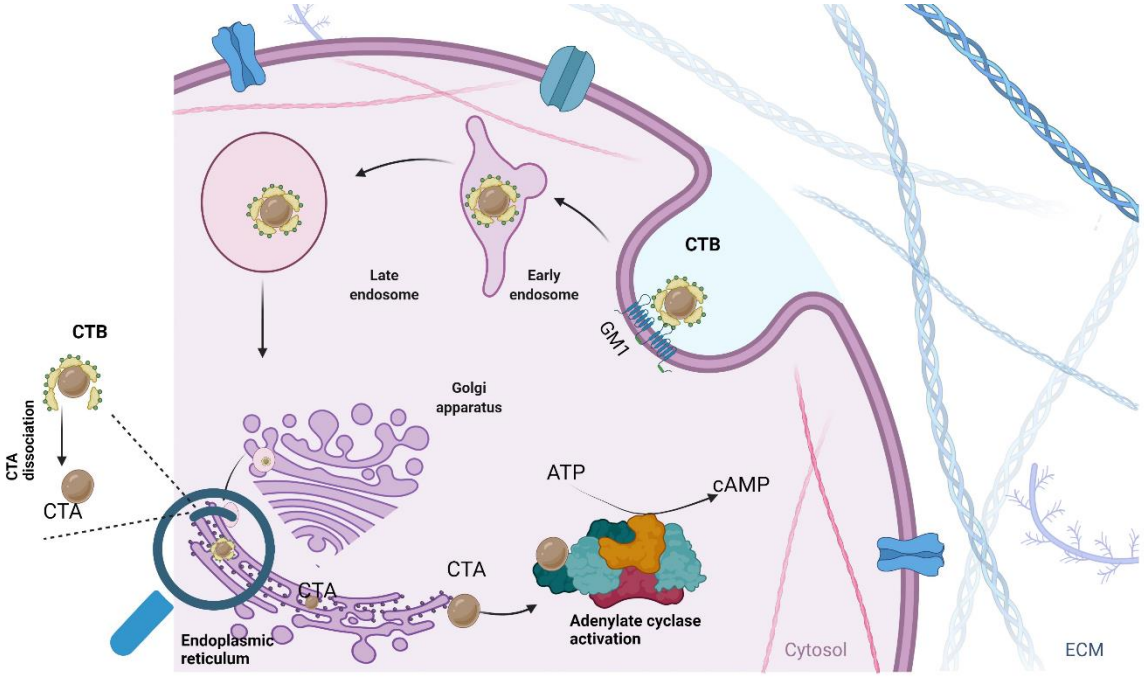

Figure 4. The current paradigm for the entrance and poisoning of CT, CT's binding to the ganglioside GM1 efficiently clusters the lipid on the cell surface, where it may be endocytosed through clathrin-dependent or –independent mechanisms. CT is transported to the endoplasmic reticulum (ER) through a retrograde transport route after endocytosis. CTA dissociates from CTB as it reaches the ER. CTA1 enters the cytoplasm of the cell and activates adenylate cyclase, converting ATP to cyclic AMP.

Toxins as Adjutants

Some of toxins can access to the immune system. For example, cholera toxin subunit B (CTB), because its affinity to the monosialo tetrahexosyl ganglioside (GM1), can call dendritic cells, macrophages, and B cells that express GM1 at their cell surface. Cholera toxin consists subunit A (CTA) and subunit B (CTB). Because of its non-toxic nature and also cellular distribution of its receptor, CTB is an ideal delivery vehicle.[107] Through pentamers formation, CTB makes a ring-like structure that receives only one CTA, resulting in an AB5 hexameric structure. After CT binds to the monosialo-ganglioside GM1 as own specified receptor, depending on cell type, either by caveolin-coated vesicles, clathrin-coated vesicles, or by the so-called Arf6 endocytic pathway is internalized to the cell. After endocytosis, CT transport to the endoplasmic reticulum (ER) via a retrograde transport pathway. After reached the ER, CTA dissociates from CTB.[108] The CTA1 enter to the cell cytosol and activates adenylate cyclase, which converts ATP to cyclic AMP. Finally, it prompts a deadly loss of water by the digestive tract (Figure 4).[107] Several approaches have been applied for using CTB as an adjuvant. In one of them, CTB recombinantly can be linked with the desired protein. Previously, CTB was linked either with *Streptococcus* protein G C-terminal, N-terminal or both, and as a result, the fusion of protein G C-terminal with CTB gave the best pentamer formation and revealed the highest binding to the GM1 receptor. In a different form, CTA1 replaced the adhesin from *Streptococcus* and so, the CTB was co-expressed but unfused with adhesion. In this method, CTB pentamer will form and CTA2 takes out adhesion from the ring structure formed by CTB**.** In another approach, CTB has not been genetically linked to the Ag, so that it is accompanied by the merozoite *Plasmodium yoelii*-derived surface protein-1 by CTB-derived N-linked oligosaccharides. As a result of such cooperation, challenge with *Plasmodium*-protected mice.[109] Also, as a DNA vaccine, CTB fused to ovalbumin acts as a good adjuvant.[110]

The Tetanus Toxoid serves as a memory antigen to activate peripheral blood mononuclear cells (PBMC). Tetanus Toxoid is employed as a carrier protein in glycoconjugate vaccines, functioning as a vaccine adjuvant that triggers protective immune responses. The heat-labile toxin (LT) of *Escherichia coli* is powerful triggers for the immune system, leading to the production of antigen-specific secretory IgA and IgG and IgA antibodies in the blood. Additionally, these toxins can serve as enhancers for boosting antibody responses in both mucosal and systemic

compartments when co-administered with protein antigens.[111] Shiga toxin (Stx) derivatives, such as the Stx1 B subunit (StxB1), which mediates toxin binding to the membrane, and mutant Stx1 (mStx1), which is a nontoxic doubly mutated Stx1 harboring amino acid substitutions in the A subunit, possess adjuvant activity via the activation of dendritic cells (DCs).[112]

Viral Infections

In spite of advances in understanding of HIV type 1 pathogenesis, including important virus components inducing the immune responses, recombinant vaccines, and treatment protocols, obstacles stay in the battle against HIV-1.[6,113-115] Hence, attitudes have recently shifted to the use of toxin-based therapies, especially bacterial toxins, due to their interference with the production and neutralization of infectious HIV-1 particles. The results of several studies show that use of bacterial toxin alone or in combination with peptides or antibodies, have made significant progress and have the potential to be effective in the future for a wide range of viral diseases (Table 2). Immunotoxins are recombinant fusion proteins composed of a toxin conjugated to a portion of an antibody, which target proteins present in virus-infected cells. The fusion protein can be linked to a diverse range of toxins, including fungal, bacterial, or plant toxins. After cell selection by antibody motif, toxin promotes *cell death* by shutting down protein synthesis *or inducing* the *breaks* in double-stranded *DNA.*[121]

Table 2. Bacterial Toxins Targeted Virus-Infected Cells

Bacterial toxin	**Target**	**Toxic moiety**		**Mechanism**	**Virus type**	**year**	**Ref**
PTX	chemokine receptor CCR5	Pertussis toxin		Inhibitory effect on HIV-1 entry into primary T lymphocytes through desensitization of CCR5	HIV-1	1999	(116)
CTX	intestinal epithelial cells	Cholera toxin subunit A		Reduces replication of HIV-1 in the HT-29 cell line (human colorectal) through activation of adenylate cyclase	HIV-1	2010	(117)
Peptide compounds	Target	Peptide moiety	Toxic moiety	Mechanism	Virus type	year	Reference
Penetrating Peptide-enterotoxigenic *E. coli*	Gag p24 (a major core protein)	a cationic 18 amino acids peptide against HIV-1 gag p24	Heat-labile enterotoxins (LTs) of *E. coli*	Inhibiting effect on HIV-1 virus capability to develop resistance by involvment with HIV-1 infection and replication via the peptide and toxin	HIV-1	2017	(118)
Immunotoxin	Target	Antibodymoiety	Toxic moiety	Mechanism	Virus type	year	Reference
CD4(178)-PE40	CD4-binding site of envelop proteins (gpl20 and gp41)	Anti-CD4 FV	PE40	shutting down protein synthesis and inducing death in gpl20and gp41-expressing cells	HIV-1	1998	(119)
3B3-PE38	CD4-binding site of gpl20	Anti-gp120 scFv	PE38	shutting down protein synthesis and inducing death in gpl20-expressing cells	HIV-1	1998	(120)

Drug Delivery

Considering the delivery of bacterial exotoxins into the cytosol, there has been significant interest in utilizing bacterial toxins for transporting macromolecules. For instance, Anthrax toxin has been utilized for the transportation of enzymes, and heat-labile Enterotoxin IIa was modified to transport a different protein into neurons. The translocation domain *Pseudomonas aeruginosa* exotoxin A (PE) has been employed to deliver a protein cargo using cell-penetrating peptides (CPPs) to aid in escaping the cargo from endosomes.[122] Similarly, Shiga-like toxin II (SLT-II) from *Escherichia coli* transports its toxic domain into the cytosol via a similar pathway as PE. The B-subunit of SLT (StxB) binds to Globotriaosylceramide (Gb3) on the cell surface and follows a retrograde route from the plasma membrane to the endoplasmic reticulum (ER). Toxins that target intracellular pathways possess a “T” domain which aids in facilitating the entry of the C domain from the cell membrane into the cytoplasm. Among these toxins are diphtheria, *Pseudomonas* exotoxin A, tetanus, and botulinum toxins. This feature is used to transfer of some cytotoxic drugs like chemotherapeutics and enzymatic part of another toxin.

Therapeutic Application of Toxin–Antitoxin Systems

Disrupting the balance of toxin-antitoxin levels in bacteria can lead to cell death, with potential applications for inducing bacterial suicide and developing new antibiotics. One example is a peptide derived from CcdB that inhibits DNA gyrase, leading to bacterial death in *E. coli*. Additionally, the toxin f in the x-e-f TA system was found to phosphorylate UNAG, inhibiting peptidoglycan synthesis. The enzymatic product of toxin f, UNAG-3P, shows promise as a broad-spectrum antibiotic. Other toxins like mazF have potential applications in antiviral gene therapy, with the ability to induce cell death in infected cells. Fusion proteins containing MazF have been designed for targeted viral cell death.[66]

Engineering of Bacterial Toxins for Therapeutic Uses

Bacterial toxins are proteins with the ability to perform various impressive functions. They act as self-sufficient molecular tools, targeting specific cells to either create pores in their membranes or alter their internal components. The process of intoxication involves intricate and specialized stages. This complexity entices biochemists, protein engineers, biotechnologists, and medical scientists to harness the advanced attributes of bacterial toxins in developing novel molecules derived from toxins for applications in research, biotechnology, and medical therapies. Some toxins or their subunits retain their natural form for use as tools in biochemistry and cell biology, or for treating specific diseases. For example, streptolysin O (SLO) is utilized to transiently puncture cell walls to deliver oligonucleotides or proteins to the cytoplasm. The Cholera toxin B pentamer is employed as a marker of lipid rafts due to its specific binding to gangliosides found in these membrane micro domains. It also serves as an enhancer in mucosal vaccines. *Clostridium* toxins help in the examination of actin and G proteins, while botulinum toxin aids in managing dystonias. Additionally, natural bacterial toxins deactivated through chemical processes are used in vaccines, including those against diphtheria, tetanus, and pertussis.[123]

Conclusion

Toxins are the most pathogenic factors produced by many bacteria involved in severe diseases in humans and animals; they can be applied as effective tools for treatment and prevention. Nowadays, important progress in the understanding of the molecular and cellular mechanisms of action of bacterial toxins has led to their applying against many cancer types. Previously, the drugs based on natural toxins like diphtheria had been used for the treatment of a blood cancer (Cutaneous T-cell lymphoma). In recent decades, new engineered toxins with minimum adverse effects have been designed against solid tumors. In this review, we tried to overview of bacterial toxins pathogenicity and their application in prevention and treatment. Nevertheless, there are other bacterial toxins that are pathogenic or usable for different goals; but we have only mentioned the most important ones.

Future Prospects

In the past, microbial toxins were known just as dangerous substances released from bacteria against other bacterial or host cells. But with the advancement of molecular and chemical science in the field of the use of toxins in medical applications, those perceptions changed completely. So that today, the science of cosmetic surgery is completely unimaginable without the use of BOTOX. Also, these bacterial substances are applicable in orthopedics, ophthalmology, and drug development in the field of cancer treatment.[121] We believe that in the near future, people will forget that bacterial toxins can be harmful. Also, the usage of bacterial toxins will not be limited to the applications mentioned in this review and their use will increase, especially in the field of saving human lives.

Authors' Contributions

ER, PS, AAIM, and HS conceived the study and designed experiments. ER, MK, MK, HS and YSH wrote the manuscript with support from all authors. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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