Bacterial Membrane Vesicles

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Pathogenic bacteria interact with cells of their host via many factors. The surface components, i.e., adhesins, lipoproteins, LPS and glycoconjugates, are particularly important in the initial stages of colonization. They enable adhesion and multiplication, as well as the formation of biofilms. In contrast, virulence factors such as invasins and toxins act quickly to damage host cells, causing tissue destruction and, consequently, organ dysfunction. These proteins must be exported from the bacterium and delivered to the host cell in order to function effectively. Bacteria have developed a number of one- and two-step secretion systems to transport their proteins to target cells. Several authors have postulated the existence of another transport system (sometimes called “secretion system type zero”), which utilizes extracellular structures, namely membrane vesicles (MVs).

membrane vesicle virulence factors secretion systems pathogenesis bacterial toxins

1. Introduction

The first report of bacterial membrane vesicles appeared in the mid-twentieth century [1]. In this study, the protein exotoxin secreted by *Vibrio cholerae* was shown to be resistant to proteases. Transmission electron microscope (TEM) analysis suggested that this exotoxin is located within spherical structures containing components of the bacterial cell envelope. These structures, detected in cell-free supernatants obtained from liquid bacterial cultures in the exponential growth phase [2], were named membrane vesicles (MVs).

As enveloped structures, MVs have the characteristics of vectors that enable the transport of substances highly sensitive to environmental conditions. They protect proteins enclosed in their lumen against enzymatic decomposition, degradation related to low or high pH and oxidative stress conditions. Therefore, it is not surprising that, in addition to proteins acquiring of nutrients from the environment, pathogenic bacteria also use MVs to transport toxins that directly affect host cells and enzymes promoting bacterial colonization, facilitating the disruption of infected tissues and spreading of infection in the host. We provide examples of the best characterized bacterial virulence factors associated with MVs in Table 1. The enrichment of certain proteins in MVs, at a higher concentration than found in bacteria, suggests a degree of specification for MVs in toxic activity, polymer decomposition, antibiotic inactivation or metal ion sequestration. The small size of MVs (ranging from 20–250 nm in diameter) [3] permits them to overcome epithelial barriers, such as the gut–blood barrier (GBB), and enter tissues that are not colonized by the bacteria producing them. The presence of surface antigens allows MVs to interact with cells of the host immune system, so that virulence factors they transport can modulate (induce or inhibit) the immune response. MVs can also act as “traps” for antibodies circulating in the inhabited tissue, or for bacteriophages in the natural environment. The great versatility of vesicles is the result of variation in their structure

and composition. The secretion of active factors in this form is one of the most complex and diverse mechanisms of bacterial interaction with the environment and other cells [4].

2. Structure of Membrane Vesicles (MVs) and Mechanisms of Secretion

The production of MVs (both extracellular and intracellular) has been observed in organisms from all three domains of life [5]. Research on bacterial vesicles has been ongoing for over 60 years, but the mechanisms of their biogenesis are still not fully understood. Several vesicle types have been described in Gram-negative and Gram-positive bacteria. The MVs exhibit the membrane features of the originating bacteria and thus could indicate the nature of their cargos, such as proteins and nucleic acids (**Figure 1**).

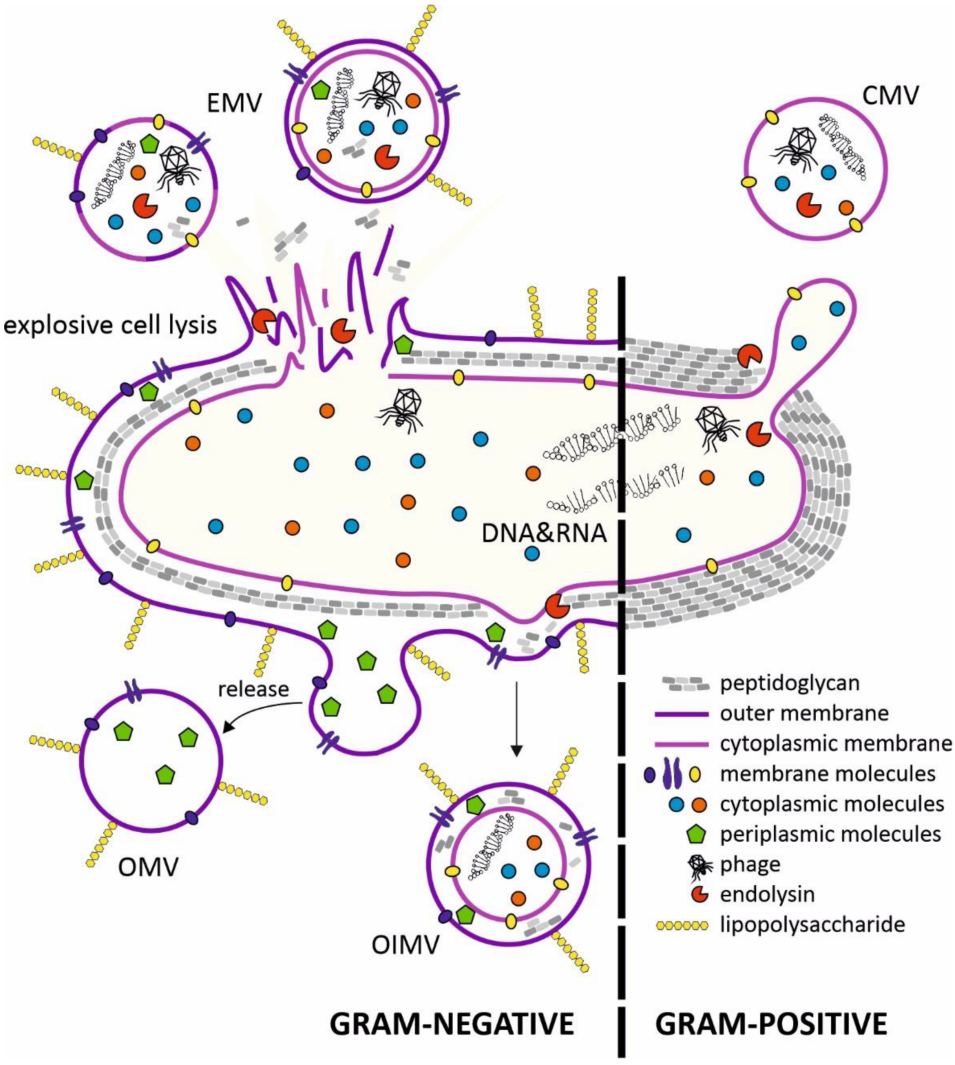

Figure 1. Mechanisms of bacterial membrane vesicle formation. In gram-negative bacteria, membrane vesicles are produced through membrane blebbing or explosive cell lysis triggered by phage-derived endolysins. Endolysins participate in the formation of cytoplasmic membrane vesicles (CMVs) in Gram-positive bacteria. The cytoplasmic membrane protrudes through holes in the peptidoglycan degraded by phage-derived endolysins. The contents of

the membrane vesicles depends on the route of their formation. EMV—explosive membrane vesicle; OIMV—outer-inner membrane vesicle; OMV—outer membrane vesicle; CMV—cytoplasmic membrane vesicle.

OMVs (outer-membrane vesicles) produced by Gram-negative bacteria consist of blebs of bacterial outer membrane containing transmembrane proteins and LPS, with extracellular DNA (eDNA) exposed on the surface of OMVs, with periplasmic content packaged in the lumen of the vesicle. OMVs are produced by many species of pathogenic bacteria, including *Neisseria meningitidis*, *Helicobacter pylori*, *Escherichia coli* (EHEC) and *Salmonella* spp. [6]. Increased secretion of OMVs usually occurs under stressful conditions, and is accompanied with the accumulation of misfolded proteins in the periplasm. According to one MV biogenesis model, the pressure of these defective proteins on the inner surface of the OM is responsible for bulging of the membrane and its detachment from the cell in the form of vesicles [7].

Outer-inner membrane vesicles (OIMVs) are double-membrane structures and were first observed in cultures of *Pseudomonas aeruginosa* [8]. The outer membrane and inner membrane are separated with a thin layer of periplasm with degraded fragments of peptidoglycan. The production of OIMVs are induced in stressful or adverse situations. Cytoplasm present in the lumen of these vesicles contains proteins and also fragments of DNA derived from the chromosome or plasmids and ATP [8].

Vesicles containing cytoplasm are also produced by Gram-positive bacteria. The release of CMVs (cytoplasmic membrane vesicles) requires local peptidoglycan degradation by internal or external lytic enzymes (digesting both the glycan backbone and peptide bonds in the amino acid chains) [9]. CMV production has been observed in several model Gram-positive bacteria, including *Bacillus subtilis* [10], *Bacillus anthracis* and *Staphylococcus aureus* [11][12].

The last membrane vesicle type is EMVs (explosive membrane vesicles), which are the most diverse in terms of structure. They arise spontaneously during bacterial cell lysis. Fragments of membrane, together with the outflow of cytoplasm (also periplasm in the case of Gram-negative bacteria), create spherical membrane structures in the environment. Thus, the process of EMV “assembly” is cell-independent and spontaneous, so bacteria are unable to control the content of these vesicles. As a result, each lysing cell produces MVs that differ in size, composition and function. This process has been described in *P. aeruginosa* biofilms, where deeply located cells subjected to hypoxia, nutrient deficiency and activation of the SOS system are autolysed through the activity of endolysins, and type R and F pyocins [13]. EMVs released in this way are an important factor in the virulence of pathogenic *P. aeruginosa* strains. As a component of the biofilm matrix, they bind eDNA and polysaccharides, and also bacteria via surface adhesins, which stiffens this structure [14].

Several recent reviews describe the composition and biogenesis of bacterial membrane vesicles [6][7][15][16][17]. In this article we present the latest data concerning interactions between MVs and selected human cell types.

3. Conclusions

The molecular understanding of bacterial virulence factors is an important challenge for microbiologists. Modern techniques have enabled the discovery of novel mechanisms that sometimes surprise researchers with their universality. This is the case for membrane vesicles, which play important roles in the interactions of bacteria with cells of other organisms. MVs are not only a new type of secretion system, their great variety of structure and function, action at a distance, and stability in the host system make them an important weapon in the bacterial arsenal. Examples of the best characterized bacterial virulence factors associated with MVs are presented below in **Table 1**.

Table 1. Examples of bacteria producing membrane vesicles and active factors discovered inside/outside MVs.

Bacterial Species (Gram-Negative)	Active Factors	Reference
Acholeplasma laidlawii PG8	• adhesins/invasins—enable tight physical contact between bacterium and host cell • ABC transporting complexes • hydrolases: proteases, nucleases, and glycosylases • metallo-β-lactamase	[18]
Acinetobacter baumannii	• AmpC—β-lactamase • OmpA—porin with potential cytotoxic features	[19]
Actinobacillus pleuropneumoniae	• Apx—exotoxin with cytolytic features	[20]
Aggregatibacter actinomycetemcomitans	• leucotoxin (Ltx)—induces lysis of monocytes and neutrophils • AbOmpA—porin that enables transport of soluble substances in MV lumen across membrane	[21]
Bartonella henselae	• HbpC—protein accumulating hemin; hemin sequestration protects bacteria from toxic concentrations of this porphyrin	[22]
Borrelia burgdorferi	• enolases—enzymes cleave plasminogen to plasmin active form	[23][24]

	• OspA/B/D—lipoprotein; promotes adhesion of OMVs to host cells (especially cells of endothelium)	
Burkholderia cepacia	• spreading factors—non-specific lipases and proteases (including metallo-proteases)	[25]
Campylobacter jejuni	• CDT—three-component genotoxin (CdtA/B/C) with endonuclease features, stops cell cycle at G2/M phase check-point	[26]
Coxiella burnetti	• periplasmic effector proteins	[27]
Escherichia coli K1	• OmpA—interaction with Ecgp receptor on surface of brain microvascular endothelium leads to cell invasion; may also act in trans to promote cell invasion by other bacterial species • K1 antigen—polysaccharide antigen from cell envelope, linear polymer of NeuNac • TLR ligands—flagellin, lipoproteins, poly-CpG DNA strands	[28]
Escherichia coli O157: H7 *Shigella dysenteriae*	• Shiga toxin (Stx1/2)—toxin from AB5 group with RNA-N-glycosylases activity; stops eukaryotic translation	[29]
enterotoxic *E. coli* (ETEC)	• thermolabile toxin (LT)—activates adenylate cyclase to elevate cAMP levels which disturbs water management of host cell; form linked to OMVs may also be non-febrile adhesin	[30]
enterohemorrhagic *E. coli* (EHEC)	• ClyA—pore-forming cytolysin; reducing environment of OMV lumen promotes ClyA oligomerization to produce active complex • HlyA—alpha-hemolysin; damages enterocyte mitochondrial membranes	[31]

extraintestinal pathogenic *E. coli* (ExPEC)	• HlyA—alpha-hemolysin • CNF1—cell necrosis factor	[32]
Haemophilus influenzae type B (Hib)	• LPS and other strong surface antigens • proteins that assist in process of biofilm formation	[33]
Legionella pneumophila	• Map—acidic phosphatase • ProA1—metallo-protease • LasB—elastase • legionaminic acid—component of LPS O-antigen • inhibitors of phagosome-lysosome fusion	[34]
Moraxella catarrhalis	• MID—protein linking IgD, superantigen • UspA1/2—blocks C3 protein of complement system • Bro1/2—beta-lactamase	[35]
Neisseria meningitidis serogroup B	• PorA—main surface antigen of OMVs; potential component of future vaccine • LpxL1—strong adjuvant	[36]
Porphyromonas gingivalis	• gingipains—non-specific proteases degrading elements of host's tissue and cytokines • HmuY—lipoprotein accumulating heme; assists biofilm formation process • factors assisting in co-localization with *Treponema denticola*	[37]

Salmonella enterica	• SopB—protects SCV (*Salmonella*-containing vacuoles) from degradation by reorganization of actin cytoskeleton • SipC—protein assisting in cell invasion process • SopA—ubiquitin ligase (E3) disturbing ubiquitin pathway of host cell • FljB—flagellin, strong antigen • SopE2—guanine nucleotide exchange factor (GEF); by catalysing exchange GDP → GTP disturbs function of Rho-protein family GTPases controlling dynamics of host cell cytoskeleton, which leads to membrane surface deformation and assists invasion process • PagK1/2—exact function still unknown; probably assists bacterial proliferation inside SCV • SrfN—promotes bacterial survival inside macrophages	[38]
Shigella flexneri	• IpaD—controls cell invasion process • IutA—iron-siderophore receptor	[39]
Treponema denticola	• dentilisin—protease	[40]
Vibrio cholerae	• cholera toxin (CTx)—AB_5 group toxin; disturbs ion-transfer across cell membranes and water management	[41]
Yersinia pestis	• Ail—surface adhesin; promotes contact with host cells • Pla—extracellular protease; activator of plasminogen • Caf1—fimbrial antigen F1; main component of OMVs	[42]
Bacterial Species (Gram-	**Active Factors**	**Citations**

Positive)		
Bacillus anthracis	• anthrolysin (ALO)—cholesterol-dependent cytolysin • lethal factor (LF)—zinc-protease; hydrolyses several MAPK-kinases (MAPKK), causes disturbance of signalling pathways and cell death • edema factor (ED)—calmodulin- and Ca^{2+}-dependent adenylate cyclase; induces uncontrolled increase in cAMP concentration in phagocytic cells thus depleting ATP reserves	[12]
Clostridium perfringens	• N-acetyloglukozamina — ważny czynnik prozapalny	[43]
Enterokok faecium	• fosfolipidy; zmniejszyć działanie przeciwbakteryjne antybiotyku daptomycyny • SdrD — białko wiążące kolagen • PavA — białko wiążące fibronektynę • AtlA – autolizyna; wspomaga proces powstawania biofilmu • Acm — MSCRAMM (składniki powierzchni drobnoustrojów rozpoznające cząsteczki matrycy adhezyjnej) z grupy adhezyny; wiąże kolagen • Fnm — adhezyna wiążąca fibronektynę • PsaA – lipoproteina; potencjalny składnik przyszłej szczepionki	[44]
Prątek gruźlicy	• LpqH — lipoproteina; asystuje w procesach transportowych • MPB83 — wysoce immunogenna glikoproteina • LprA — lipoproteina; silny agonista TLR2 • PSTS3 — element systemu transportowego ABC związany z importem jonów fosforu	[45]

	• lipoarabinomannan (LAM) — glikolipid powierzchniowy o właściwościach anty-ROS • mykobaktyna – powierzchnia Fe^{3+} -syderofor	
Propionibacterium acnes	• czynniki aktywujące zapalenie zależne od TLR2	[46]
Streptococcus mutans	• eDNA — ważny składnik biofilmu • glukozylotransferazy (GtfB/C/D) — wytwarzają adhezyjne zewnątrzkomórkowe polisacharydy z substratu sacharozy • kwas lipotejchojowy (LTA) – antygen powierzchniowy; ważny w procesie adsorpcji w tworzeniu biofilmu	[47]
Streptococcus pneumoniae	• TatD — nieswoista DNaza umożliwiająca degradację NET (sieci DNA związane z białkami o działaniu przeciwdrobnoustrojowym: LL37, mieloperoksydaza, elastaza neutrofili) • EndA — nieswoista DNaza zlokalizowana na powierzchni MV • PspC-białko wiążące czynnik H; blokuje alternatywny szlak dopełniacza • pneumolizyna (Ply) – egzotoksyna o właściwościach cytolitycznych • PsaA – adhezyna; silny antygen powierzchniowy • SatA — transporter typu ABC; antygen powierzchniowy • AmiA — białko wiążące peptydy; asystuje w aktywnym transporcie • MalX — białko wiążące maltozę i maltodekstrynę • PnrA — transporter nukleozydów typu ABC • spr1909 — białko wiążące penicylinę	[48]

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