Review Article

New insight into the application of outer membrane vesicles of Gram-negative bacteria

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ABSTRACT

This review presents a brief outline of our current knowledge of the structure and chemical composition of the outer membrane vesicles (OMVs), originating from the surface of Gram negative bacteria including their outer membrane proteins and lipopolysaccharides. Moreover, the functional roles and applications of OMVs in medical research such as OMV-based vaccines, OMV adjuvants properties, OMV carriers in conjugated vaccines as well as in drug vehicles and delivery systems are discussed. Finally, new applications of these macromolecules as biotechnological tools are briefly presented.

KEYWORDS: OMV, vaccine, drug vehicles, delivery systems.

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INTRODUCTION

Outer membrane vesicles (OMVs) are small spherical structures, approximately 20–250 nm in diameter which are produced by the growing cells during all phases of the bacterial growth, rather than being a consequence of the cell death [1]. OMVs could be produced in a variety of environments including liquid and solid cultures, biofilms as well as during bacterial stress conditions. These macromolecules consist of two membranes, namely the inner and the outer membranes (phospholipids), outer membrane proteins (OMPs), peptidoglycan layer, lipopolysaccharides (LPS) and the periplasm components. Thus, OMVs consists of the protein and lipids of the outer membrane and the periplasm. Both pathogenic and nonpathogenic species of Gram-negative bacteria secrete OMVs. In general, pathogenic bacteria produce more OMVs than their nonpathogenic counterparts. For instance, enterotoxigenic Escherichia coli (ETEC) produce nearly 10 times more OMVs than the nonpathogenic E. coli. Similar patterns in OMV production occur for leukotoxic and nonleukotoxic Actinobacillus actinomycetemcomitans in which their pathogenic strains produce 25 times more vesicles [1, 2]. The Functional roles and characteristics of OMVs are summarized as follows.

SECRETION AND DELIVERY SYSTEM

OMV secretions consist of lipids, membrane proteins and other insoluble bacterial compounds. For example, the vesicles produced by standard cultures of Pseudomonas aeruginosa and E. coli account for ~1% of the outer membrane components in these cultures. In contrast, Neisseria meningitidis produces high amounts of the vesicles, constituting of 8 to 12% of the proteins and endotoxins produced in the log-phase of the culture. OMVs of the pathogenic bacteria could be served as tools for secretion of toxins, enzymes and virulence factors. These vesicles mediate delivery of such compounds by lysing and attaching to the target spontaneously and delivering their contents by proximal lysis, internalization or fusion processes [1, 3].

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OMV-ENABLED BACTERIAL SURVIVAL

Defense and resistance against antibacterial compounds could be performed by releasing of OMVs in all stages of the bacterial growth. For instance, OMVs production can quickly remove a surface-attacking agent from the bacteria and their production can also increase the survival rate of the bacteria when attacked by the lytic phages. OMVs can also absorb antibacterial entities, such as complements and antibiotics [1-3].

NUTRIENT ACQUISITION

Some OMVs have been found to contain scavenging proteases, namely xylanase and cellulase which can aid in nutrient acquisition and thereby provide a survival advantage for the OMV-producing bacteria [1, 4]. Finally, the secreted OMVs can play a role in pathogenesis, quorum sensing, nutrient acquisition and host parasite interactions [1, 5, 6].

APPLICATIONS OF OMVS IN CARRYING SMALL MOLECULE ACTIVATORS AND INHIBITORS OF

QUORUM SENSING IN GRAM-NEGATIVE BACTERIA

The OMVs provide a protective environment for the cargo, like quorum-sensing molecules involved in cell-cell communications such as 2-heptyl-3hydroxy-4-quinolone (Pseudomonas quinilone signal or PQS). Interestingly, it is believed these OMVs carrying quorum-sensing molecules were able to participate as activators and inhibitors of quorum sensing. Therefore, OMVs are capable of carrying and secreting bioactive compounds [4, 7]. Furthermore, the genomic DNA found in the OMVs has been successfully transferred into other bacterial cells which may constitute a new DNA delivery system [2, 8].

GENETIC ENGINEERING OF BACTERIAL OMVS

One of the advantages of OMVs, is the possibility of embedding functional proteins such as green fluorescence protein (GFP) and β-lactamase (Bla) on their surface,via the virulence factor cytotoxin ClyA as the surface anchor (e.g. in hyper-vesiculating E. coli). This indicates the feasibility of designing OMVs as synthetic nanoreactors by using only standard molecular biology techniques [10].

MICROBIOTA OMVS

The role of OMVs inside human microbiota has received attention in recent years. This role is not only focused on their ability to carry a wide variety of enzymes for polysaccharides (PS) digestion, but it also points to the immunomodulation properties of OMVs. For instance, Bacteroides fragilis OMVs carrying polysaccharide A (PSA) could be sensed by Toll-like receptor 2 (TLR2) of the dendritic cells which induce growth arrest and the production of DNA-damage-inducible protein (Gadd45a). Such OMVs can also increase IL-10 production from Foxp3+ iTreg cells. This ability of the microbiota OMVs due to their vehicle properties are considered important in human gut modulation and development [11]. Another interesting example of the OMV role in microbiota-host communication is Bacteroides thetaiotaomicron OMVs where an enzyme involved in intracellular Ca2+ signaling and with implications in cancer (active homologue of the eukaryotic inositol phosphate phosphatase) has been detected [3,11]. In conclusion, the OMV-based network is likely to be involved in important relationships in order to create organized ecological balance within the intestinal microbiota [9].

IMMUNOMODULATORY ACTIVITIES

The composition of OMVs makes them significant activators of host innate and acquired immune response pathways. In addition to the potent immunomodulatory molecule LPS, OMVs contain OMPs and other important innate immune-activating ligands. Together, vesicle components could act synergistically to modulate the host response in ways that they can either stimulate the clearance of the pathogen, enhance the virulence of the infection, or both. In addition, the immunogenic properties of OMVs lead to protective mucosal and systemic bactericidal antibody responses that have been exploited for the vaccine purposes. OMVs from Salmonella enteric serovar Typhimurium are stimulators of proinflammatory cytokine secretion and immune cell activation [3, 12]. Salmonella OMVs activate macrophages and dendritic cells to increase surface MHC-II expression levels as well as the production of the proinflammatory mediators TNF-α and IL-12. OMVs also activate CD4+T cells [3, 12, 13].

A PROINFLAMMATORY RESPONSE

Helicobacter pylori OMVs elicit an IL-8 response, similar to P. aeruginosa vesicles. Detergent-generated vesicles from N. meningitidis have been shown to trigger the production of numerous proinflammatory cytokines from PMNs, including TNF-α, IL-1β, IL-8, MIP-1β and IP-10 [11, 12, 14].

OMV-BASED VACCINES

Studies of the host immune responses to OMVs have mainly addressed the generation of antibodies to the vesicle components. Notably, a protective antibody response is elicited by OMVs generated from N. meningitidis, Vibrio cholera, Bordetella pertussis, Francisella tularensis, Brucella spp., Acintobacter spp., Shigella spp., Salmonella spp. and Borrelia burgdorferi [15-19]. All preparations of Neisseria vesicle vaccines have been shown to stimulate protective mucosal and systemic bactericidal antibody responses while the generated antibody responses are predominantly toward the outer membrane porins such as PorA and PorB. Research is currently focused on engineering bacterial strains to produce OMVs containing multiple PorA proteins derived from different strains in hopes of developing a global N. meningitidis serogroup B vaccine [16, 20-22].

UNIVERSAL ADJUVANT PROPERTIES OF OMV

Several studies have reported the adjuvant properties of OMVs in the field of vaccine research, namely in cancer vaccines, brucellosis vaccine, tuberculosis (TB) vaccine, meningococcal vaccine, Influenza virus vaccine, hepatitis B vaccine and human immunodeficiency virus (HIV) vaccine. [12, 13, 21, 23-25]. Most of the classic and new adjuvants cause local and systemic hypersensitivity reactions and are not licensed for human use. Due to these drawbacks of the current adjuvants, OMVs could be considered as an alternative safe adjuvant with a high potency to induce typical secondary immune responses. OMVs have been previously used in vaccine formulations in human trials and were found to be safe. Several reports have described that PS antigens stimulate immunologic memory when combined to OMV which have been shown to have T helper mitogenic activity [13, 24-26]. Thus, the availability of such OMV component with adjuvant properties will be of great importance for the development of improved and combined vaccines for a wide variety of diseases. In addition, the adjuvant properties of OMV-derived particles have been demonstrated for potential cancer vaccines [25, 27].

Interesting studies in the field of brucellosis subunit vaccines have been carried out by Bhattacharjee et al. and Sharifat et al. [23, 28] which have evaluated group B OMPs (GB-OMV). Brucella melitensis strain 16M LPS non-covalent complex to elicit the immunity against brucellosis in mice. In this study, they have extracted LPS from B. melitensis and applied OMVs of N. meningitidis as an adjuvant [33]. Furthermore, in order to explore the efficacy of Brucella abortus, LPS combined with different adjuvants and proteins (as a vaccine candidate) have been used which resulted in induction of effective and long-lasting immunity against brucellosis. Sharifat and colleagues have also evaluated and reported the OMVs of N. meningitidis

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serogroup B as a potent subcutaneous adjuvant and a part of a candidate vaccine against brucellosis which have been shown to induce high titers of specific anti-B. abortus S99 LPS in an animal model [21,23, 28].

OMV AS A CARRIER IN CONJUGATED VACCINES

While the adjuvant properties of meningococcal OMVs are expected, the potency of OMVs as a carrier (conjugated to a hapten) has also been proven. It has been documented that PS originated from bacterial capsules or LPS when conjugated to proteins are usually immunogenic in mice and rabbits as well as in humans. Many studies have shown that these conjugated vaccines can induce humoral and cellular immunity against many pathogens in humans including N. meningitidis, V. cholera, Haemophilus influenzae, Shigella sonnei, as well as Brucella spp. Covalent linkage of PS or LPS to carriers such as proteins produce glycol-conjugates which are T-dependent antigens and prime for long lasting immunity with either PS or LPS. On the other hand, PS or LPS-protein conjugates have been proven to be effective in several cases while well- defined glycoconjugate vaccines have also been explored with an intention to elicit discriminating immune responses [16, 21, 22, 29]. The latest study in brucellosis vaccines have employed a conjugated B. abortus LPS with GB-OMV which could induce both humoral and cellular immune responses against Brucella spp [17].

FUTURE DIRECTIONS

We could mimic the design and synthetically produce OMV or even better, use appropriate engineered bacteria which could themselves produce large quantities of secreted OMVs with a content and specificity of choice as nano-sized drug delivery vehicles [13]. Moreover, the utilization of OMVs as a complex of antigens in their native context with a natural adjuvant has already been proven to be successful for human vaccines. The presence of LPS in OMV-based vaccines has emphasized the ability of LPS to act as a natural adjuvant for the immune system. Future efforts will likely result in OMV vaccines engineered to reduce the endotoxicity and to include multispecies-specific antigens against pathogenic bacteria such as N. meningitidis and Acinetobacter baumannii. In this regard, an interesting study has been reported recently in which OMV-generating bacterial mutants (E. coli) with low endotoxicity have been produced [30]. Thus these bacteria could be used as a safe expression system to produce recombinant proteins such as vaccines candidates or enzymes. Interestingly, bacterial OMVs are evolution-driven processes that carry protein antigens in conformational states which are similar to the original structure. Thus, OMVs could be considered as a non-reacting compound which could produce recombinant proteins in future expression systems. Furthermore, OMV-biogenesis could be considered as a biotechnological tool for expression and delivery of protein antigens as well as enzymes in a wide variety of biomedical research projects [30-32].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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