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A Glimpse on Outer Membrane Vesicles as Vaccine Candidates

Leitner DR, Zingl FG and Schild S*

Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50, A-8010 Graz, Austria

***Corresponding author:** Schild S, Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50, A-8010 Graz, Austria, Tel: +4303163801970; Fax: +4303163809019; E-mail: stefan.schild@uni-graz.at

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Description

Recently it becomes increasingly evident that actively release of membrane-derived vesicles from cellular surfaces is conserved in all domains of life, including eukaryotes, archaea, Gram-positive and Gram-negative bacteria. For the latter case these spherical structures originate from the outer membrane (OM) and are consequently referred to as outer membrane vesicles (OMVs). Notably, all Gram-negative bacteria that have been investigated so far are able to naturally release OMVs [1,2]. Although OMV formation seems to be a common feature of Gram-negative bacteria the knowledge of their biological roles and biogenesis remains very limited. The hypothesized functions range from simple waste dumps to delivery vehicles for virulence factors (e.g. toxins), degradation enzymes (e.g. toxins and proteases), DNA to enable horizontal gene transfer or signaling molecules for intra- and inter-species communication (e.g. quorum sensing signaling molecules) [3,4]. OMVs range from 10-300 nm in diameter and consist mainly of OM components, such as phospholipids, OM proteins, and lipooligosaccharide (LOS) or lipopolysaccharide (LPS). Additionally, OMVs contain periplasmic components, which are trapped in the lumen of OMVs during the vesiculation process [5]. Hence, OMVs are basically non-living facsimiles of the bacterial cell surface, naturally containing multiple native surface-exposed antigens as well as immunostimulatory molecules [1,6]. Based on their aforementioned immunogenic potency and on positive examples of the OMV-derived vaccines against serogroup B *Neisseria meningitides*, we initiated several projects over the last years to analyze the potential of OMVs derived from human Gram-negative pathogens as vaccine candidates [7-12]. This concise communication summarizes our recent findings in the emerging research field of OMVs as vaccine candidates including immunization studies against human gastrointestinal pathogens (e.g. *V. cholerae* and ETEC) as well as human and veterinarian respiratory tract pathogens (e.g. *Pasteurellaceae* family members).

For example, we comprehensively characterized OMVs derived from *V. cholerae* as a new approach for an effective vaccine candidate against cholera [7-10,13]. As it turns out OMVs exhibit several advantages compared to other vaccines. For example, OMVs are highly stable, even at room temperature, and highly immunogenic without requirements of additional adjuvants [7-9]. Thus, a cold chain or accessory buffer solutions are unlikely to be required for the OMV vaccine candidate. In addition, OMV donor strains can be genetically modified to secrete altered OMVs. We successfully applied this strategy to isolate OMVs from a LPS acyltransferase mutant strain, demonstrating that reduction of OMV endotoxicity can be achieved without diminishing the immunogenic potential by genetic modification resulting in underacylated lipid A [10]. Overall, we demonstrated the induction of a specific, long-lasting, high-titer, protective immune response upon mucosal immunization of mice with *V. cholerae* OMVs [7,8]. Interestingly, throughout our studies protection against O1 and O139, representing the two clinically relevant serogroups of *V. cholerae*, was only achieved by immunization with a mixture of O1 and O139 OMVs. Since serogroup O139 has evolved from serogroup O1, the only relevant discrimination marker is the variable O antigen of the LPS. Indeed, a recent study pinpoints the O antigen to be the essential immunogenic structure of the OMV based cholera vaccine candidate and provides a protective mechanism based on inhibition of motility, which prevents a successful colonization [10]. As *V. cholerae* possesses a single polar flagellum that is covered by an OM sheath including LPS molecules [14], only antibodies directed against the O antigen are efficiently blocking motility and mediate the observed protection. Strikingly, cross-protection against O1 and O139 was so far only achieved by including serogroup specific LPS in the immunization for all cholera vaccine candidates tested [15-17]. Moreover, we were able to expand the current protection model of *V. cholerae* to enterotoxigenic *Escherichia coli* (ETEC) by demonstrating that the inhibition of motility via anti-FliC antibodies is a relevant protection mechanism of an OMV-based ETEC vaccine candidate [13]. Thus, the proposed model of inhibition of motility might be a common mechanism important for several vaccine candidates against gastrointestinal pathogens.

Recently, the studies on OMVs as vaccine candidates were extended to members of the *Pasteurellaceae* family comprising human and veterinarian pathogens of the respiratory tract (e.g. non-typeable *Haemophilus influenzae* NTHi, *Pasteurella multocida and Mannheimia haemolytica*) [11,12]. A common feature of these pathogens is a significant surface heterogeneity with diverse OM protein profiles and highly variable regions in many proteins. This might be one explanation why currently no or only narrow-range protective vaccines are commercially available. Intranasal administration of NTHi OMVs induced a robust and complex humoral and mucosal immune response in the murine model [12]. In contrast to the serogroup-specific protection observed in the *V. cholerae* studies, immunization with NTHi OMVs resulted in a cross-protective immune response reducing colonization of heterologous NTHi strains. Notably, this was even true for highly heterologous strains whose OMVs were not part of the immunization mixtures. Immunoprecipitation revealed several immunogenic proteins, which are highly abundant in many NTHi strains and have at least some conserved domains (e.g. the heme utilization protein, protective surface antigen D15, heme binding protein A, and the OM proteins P1, P2, P5 and P6). Similar observations were made by using OMVs from *P. multocida* and/or *M. haemolytica*, representing two closely related veterinarian pathogens, whereby mice immunized with *M. haemolytica* OMVs exhibited protection against *P. multocida* [11]. In summary, the results of these studies indicate that OMVs of *Pasteurellaceae* family members allow the induction of cross-protective immune responses based on conserved OM proteins or at least conserved epitopes.

The presence of several antigens in their native conformation may support such cross-protective immune responses upon immunization with OMV vaccine candidates. In contrast, antibodies induced by whole cell killed vaccines rather target protein sequences than native conformational epitopes. Although whole cell killed vaccines represent so far the only commercially alternative against cholera infections, they possess several disadvantages compared to OMVs in terms of quality control, heat stability and storage conditions. Alternatively, subunit vaccines could be used due to their admirable safety record [18], however they are likely more expensive and time-consuming due to the expression and purification of single antigens. In contrast, OMVs can be easily isolated via consecutive filtration and centrifugation steps without requirement for complex buffer solutions or cold chain distribution. The recent studies also indicate that OMV mixtures from different donor strains are applicable and can extend the immune response to create a broad spectrum OMV vaccine against several pathogens of interest. Importantly, OMVs are a delivery vehicle of antigens in their native conformation, which is contradictory to most other vaccine candidates.

The potential of OMVs as vaccine candidates has now been demonstrated in a variety of studies against several Gram-negative pathogenic bacteria (for overview see [6,19]). Unfortunately, the fundamental knowledge regarding OMV biogenesis,- size, -quantity as well as their composition is still lacking. In an attempt to provide a platform for future research, a comparative characterization of OMVs and OM preparations derived from highly heterologous encapsulated and non-encapsulated *H. influenzae* isolates was performed. In this study, several independent methods, including nanoparticle tracking analysis, transmission electron microscopy, protein and/ or LOS quantification via Tray Cell, Bradford or Purpald assays were established and used to evaluate the OMV size and quantity secreted by the heterologous *H. influenzae* strains. An important outcome of these studies is that the determination of the OMV size has to be performed in parallel to the quantification measurements. For example, the OMV preparation of the encapsulated *H. influenzae* type b isolate exhibits more protein and LOS amounts compared to other isolates, which can easily be misinterpreted by hypervesiculation. However, *H. influenzae* type b OMVs are also twice as big and consequently have a larger surface area, which explains the increase in biomass. In contrast, the non-typeable *H. influenzae* 1479 exhibited similar OMV sizes compared to others but significantly increased protein and LOS amounts, indicating higher OMV production. Future studies will now focus on the biological basis for such isolate specific size differences and hypervesiculation phenotypes. Furthermore, the lipidome and proteome profile of the OM was compared with the corresponding OMVs from several heterologous *H. influenzae* strains. The lipid profile between OM and OMVs was generally very similar with a slight tendency towards more short chain fatty acids in OMVs, while the protein profile between OM and OMVs showed pronounced variation. On average, only 30% of the total identified proteins were found in the OM and the corresponding OMVs, which points towards a stringent cargo selection. Overall this study provides a landscape profile of the OMV composition in comparison to the OM in *H. influenzae*.

Only a few complimentary studies on OMV compositions of distantly related Gram-negative bacteria are currently available. Additionally, only a handful of studies have analyzed alterations in OMV composition of cultures grown under different conditions or defined mutants. In order to gain a comprehensive picture more analytical studies have to follow to uncover environmentally driven changes, common principles as well as strain- or isolate-specific differences in OMV composition. Generally our knowledge on OMV biogenesis is very limited. Is there a conserved mechanism of OMV secretion in Gram-negative bacteria? Can bacteria modulate their OMV production and control the protein or lipid sorting in OMVs? What are the important signals mediating these processes? This non-exhaustive list of questions needs to be addressed in the future to achieve a better understanding of these fascinating extracellular structures.

So far, the greatest success has been achieved with the highly efficacious anti-*N. meningitides* OMV-based vaccines, which are used in the epidemic control in Cuba, Norway and New Zealand. Currently, they represent the only licensed OMV vaccines for human use [20-22]. They demonstrate a high applicability and safety in humans [23-25]. Encouraged by these promising results several groups including us started proof of principal studies for relevant pathogens, wherein we focused on the potential of OMV based vaccines against respiratory and gastrointestinal human pathogens. Taken together, the available OMV vaccine studies indicate that the specific protective antigens and protection mechanisms are highly variable. In some cases a specific antigen has to be present to confer protection, while other examples benefit from diverse antigens present to overcome isolate heterogeneity. Important antigens range from peptidoglycan, polysaccharides to proteins. Thus, the detergent extraction method developed for the *N. meningitides* OMV-based vaccines to remove most of the LPS to lower endotoxicity is not always an appropriate method as it could result in loss of the protective antigens. Genetically modified OMV donor strains with underacylated LPS represent useful alternatives to overcome this limitation [10]. Relevant protective mechanisms include opsonization, inhibition of motility, complement activation and neutralization [26]. Thus, the molecular basis for the protective immune response needs to be solved for each OMV vaccine candidate individually.

After 20 years of the first reports of OMV-based vaccines against serogroup B meningococci several studies indicate that OMVs are a powerful and versatile tool for alternative vaccination strategies. In times of emerging antibiotic resistances, we now need to take the next steps and get the most promising OMV vaccine candidates into clinical trials to finally make them commercially available.

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