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Designer outer membrane vesicles as immunomodulatory systems – Reprogramming bacteria for vaccine delivery☆

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ABSTRACT

Vaccines often require adjuvants to be effective. Traditional adjuvants, like alum, activate the immune response but in an uncontrolled way. Newer adjuvants help to direct the immune response in a more coordinated fashion. Here, we review the opportunity to use the outer membrane vesicles (OMVs) of bacteria as a way to modulate the immune response toward making more effective vaccines. This review outlines the different types of OMVs that have been investigated for vaccine delivery and how they are produced. Because OMVs are derived from bacteria, they have compositions that may not be compatible with parenteral delivery in humans; therefore, we also review the strategies brought to bear to detoxify OMVs while maintaining an adjuvant profile. OMV-based vaccines can be derived from the pathogens themselves, or can be used as surrogate constructs to mimic a pathogen through the heterologous expression of specific antigens in a desired host source strain, and approaches to doing so are reviewed. Additionally, the emerging area of engineered pathogen-specific carbohydrate sequences, or glycosylated OMVs is reviewed and contrasted with protein antigen delivery. Existing OMV-based vaccines as well as their routes of administration round out the text. Overall, this is an exciting time in the OMV field as it matures and leads to more effective and targeted ways to induce desired pathogen-specific immune responses.

Contents

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1. Introduction

Most inactivated and subunit vaccines require the addition of an adjuvant to elicit sufficient immune responses. Improved adjuvant platforms, capable of interaction with specific pathogen recognition receptors (PRRs) of the innate immune system, can lead to more effective and longer lasting vaccines. One approach toward the generation of a pathogen mimetic adjuvant is to leverage the natural PRRs that exist in outer membrane vesicles (OMVs) derived from bacteria. OMVs naturally bud from Gram-negative bacteria, and thereby contain the pathogen-associated molecular patterns (PAMPs) present on bacterial outer membranes. These PAMPs impart immunomodulatory characteristics to the OMVs, and were recently reviewed [1]. Because OMVs are unable to replicate, there is no need to treat them with formalin or other inactivating agents, thus preserving PAMPs in their native states. Whereas bottom-up approaches use physical combinations of known PRR antagonists and antigens are formulated to immunologically mimic an invading pathogen, OMVs represent a top-down approach, starting with particles that are already pathogen mimetic. The use of OMVs as an adjuvant is research group-dependent with many innovative tactics. OMV-based vaccines may be made from the direct collection of vesicles produced by the target pathogen. Alternatively, recombinant OMV-based vaccines are created in engineered bacteria hosts that express and embed homologous or heterologous antigens from different pathogens within the OMV. While the most simplistic approach to an OMV-based vaccine is to use OMVs produced directly from the target pathogen, bacterial engineering allows for designer vaccine candidate production as well. Through gene knockout and/or transformation with plasmids, bacterial vesiculation rates can be increased, OMV lumen content influenced, lipopolysaccharides (LPS) remodeled, and foreign proteins expressed. The parameter space that can be explored is large, and OMVs represent a new and robust approach toward tailored vaccine design. This review provides an overview of how OMVs are, and can be, used as a directed immunomodulatory adjuvant system, with special emphasis on how bacteria can be genetically modified to enhance their potential as effective modulators of the immune response.

2. OMV types and OMV production

OMVs are produced from Gram-negative bacteria, although recent reports show that some Gram-positive bacteria and archaea also produce similar vesicles [2,3]. OMV biogenesis is a complex process that occurs through several different mechanisms, as was expertly reviewed elsewhere [4,5]. Bacteria produce OMVs for myriad reasons, including envelope stress relaxation, quorum sensing, defense, long-distance toxin transfer, antibiotic response and have been extensively reviewed by the Kuehn group [6–8]. When OMVs are produced naturally, the integrity of the outer membrane is not compromised; instead, it neatly buds off [9]. Though OMVs are produced naturally, they can also be produced artificially, typically through treatment of the bacteria with detergent and/or sonication. While the artificially produced OMVs are formed from the outer membrane of bacteria, their lumen contents can differ from naturally produced OMVs. Additionally, OMVs produced with detergent treatment are stripped of lipoproteins, potentially altering the desired PRR profile. There is disagreement in the OMV field over the appropriate nomenclature for OMVs made through different methods. Originally, Kuehn et al. [10] divided OMVs into native (produced naturally from native bacteria vesiculation) and non-native (produced by mechanical means). However, in a more recent review by van der Ley et al., OMVs were classified as (Fig. 1) as spontaneous 'sOMVs' (produced naturally from budding of bacteria, the equivalent of Kuehn's 'native' category), native 'nOMVs' (produced using sonication, but no detergent), and detergent 'dOMVs' (produced using detergent, thus removing some of the typical components found in a bacterial outer membrane) [11]. While the term 'native OMV' for an OMV that was produced using synthetic means is a bit confusing, we will continue to call these mechanically produced, but non-detergent treated OMVs, 'nOMVs' to remain consistent with the literature, in particular with the OMV-based vaccines associated with *Neisseria meningitidis*.

2.1. Spontaneous OMVs

Spontaneous OMVs (sOMVs) naturally bud from the bacterial outer membrane, as was first discovered in the 1960s [12–14]. Though referred to as 'spontaneous', significant research has been dedicated to

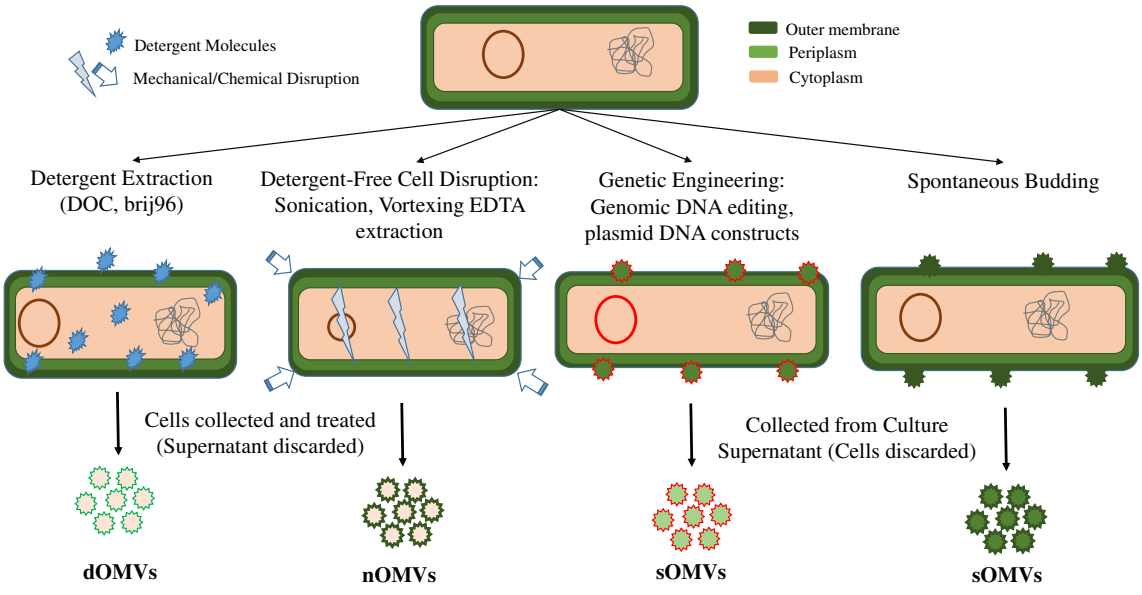

Fig. 1. OMV types nomenclature (adapted from reference [11]). dOMVs, nOMVs and sOMVs refer to detergent OMVs, native OMVs and spontaneous OMVs respectively. The color scheme in this figure aims to indicate a composition specific distribution in each OMV types. For example, sOMVs from spontaneous budding are highly composed of outer membrane proteins, phospholipids, LPS and periplasmic materials which is emulated in the color resemblance between OMVs and the bacterial cell periphery. In parallel, sOMVs from genetic manipulations could be made to express on their surface proteins genetically encoded in their plasmid or genomic DNA as represented by the bright red contour. Lastly dOMVs and nOMVs due to the method of production may contain more cytoplasmic materials in their lumen while their outer membrane may be a mix of detergent, outer membrane proteins, and phospholipids.