The Benefits of Exporting: Engineered Extracellular Vesicles as Promising Vaccine Candidates against Enteric Fever

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ABSTRACT The immunological potential of extracellular vesicles produced by Gram-negative bacteria, the so-called outer-membrane vesicles (OMVs), can be improved by genetic engineering, resulting in vesicles containing multiple immunogens. The potential of this approach for the development of a vaccine candidate for enteric fever was recently demonstrated by G. Gasperini, R. Alfini, V. Arato, F. Mancini, et al. (Infect Immun 89:e00699-20, 2021, <https://doi.org/10.1128/IAI.00699-20>). This commentary will discuss the use of OMVs to generate vaccines for enteric fever and the promise of this approach for prevention of other infectious diseases.

KEYWORDS enteric fever, extracellular vesicles, outer membrane vesicles, vaccines

The bacteremic illnesses referred to as typhoid and paratyphoid fever, known collectively as enteric fever, are caused by *Salmonella enterica* serotypes Typhi (typhoid fever) and Paratyphi A, Paratyphi B, and Paratyphi C (paratyphoid fever) (1). Globally, an annual incidence of 26 million cases of typhoid fever and 5 million cases of paratyphoid is estimated, culminating in 215,000 deaths (1). Typhoid and paratyphoid fever coexist in many geographical areas, especially in low-middle-income countries (LMIC) (2). Up to 4% of patients with acute typhoid disease can become chronic carriers (1). Antimicrobial treatment of enteric fever is threatened by the emergence of multidrug-resistant *S. enterica* strains, which led the World Health Organization (WHO) to classify fluoroquinolone-resistant *Salmonella* spp. as a high-priority pathogen presenting a risk to human health (3). Since the disease is transmitted by contaminated food or water and, occasionally, direct fecal-oral transmission, long-term prevention of enteric fever demands improved sanitation conditions with access to safe drinking water (1, 3). These conditions are not available in several regions of the world. The difficulties in treating enteric fever and controlling its transmission point to the obvious need for effective vaccines. In this complex scenario, enteric fever remains a major global health concern, and novel, affordable vaccine candidates are clearly necessary (3).

As summarized by the U.S. Centers for Disease Control and Prevention (4), there are two vaccines to prevent typhoid fever. Typhoid immunization protects 50 to 80% of recipients. *S. Typhi* is coated by a capsule that contains a key virulence factor, namely, the Vi (virulence) capsular polysaccharide (5). The Vi antigen, which is also synthesized by *Citrobacter freundii*, *S. Paratyphi C*, and *S. Dublin* (6), is the principal component of injectable typhoid vaccines (3). The typhoid Vi conjugate vaccines (TCVs) form the basis of the newest strategies to prevent typhoid fever. Recently, the WHO recommended the use of TCVs in high-burden countries (3, 7). In TCVs, the Vi polysaccharide is covalently linked to carrier proteins. The TCVs have better efficacy in inducing immune responses in children and an increased ability to induce high antibody titers than earlier typhoid vaccines, among other advantages (3).

No vaccine is available specifically for paratyphoid fever, and neither licensed typhoid vaccine prevents paratyphoid fever, although some cross-protection might

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occur (4). *S. Paratyphi* vaccines under development contain the outer repeating units of the lipopolysaccharide, O-antigen, with the conjugation of the O polysaccharide (O:2) to protein carriers (8). The O-polysaccharide is a potent immunogen that also participates in virulence (9).

Vaccine candidates combining these major antigens would be advantageous. Along these lines, the study by Gasperini and colleagues reports on the development and characterization of *Salmonella* Paratyphi A outer membrane vesicles (OMVs) as multivalent vaccine candidates for enteric fever (10). In their study, a vesicle-based, bivalent vaccine candidate delivering the Vi polysaccharide from *S. Typhi* and the somatic O antigen from *S. Paratyphi* A was produced and induced functional antibodies against both Vi and O:2 antibodies. A discussion of the conceptual basis of this promising vaccine candidate follows below.

GRAM-NEGATIVE BACTERIA PACKAGE IMMUNOGENS INTO EXTRACELLULAR VESICLES

Cells of all domains of life produce extracellular vesicles (EVs) as a regular physiological event (11). EVs are membranous components originating at multiple cellular sites in prokaryotic and eukaryotic organisms that reach the extracellular space carrying several bioactive components (12). Gram-negative bacteria spontaneously release EVs that originate at their outer membrane (13). These EVs, the so-called outer membrane vesicles (OMVs), transport surface immunogens and virulence factors in their native conformation (14). Packaging these molecules into vesicles results in the concentration of antigens into compartments that are efficiently taken up by effector immune cells, and their ability to induce protective responses has been demonstrated (15). These properties make EVs in general and bacterial OMVs specifically promising vaccine candidates (14). Indeed, OMV-containing meningococcal vaccines are available (16). Similar immunological properties were demonstrated for Gram-positive bacteria (17), fungi (18–20), helminths (21), protozoa (22), and even cancer cells (23). The use of EVs as vaccine prototypes is, therefore, of great interest to the field of vaccinology, and this has motivated the search for improved protocols for the efficient isolation of EVs (24, 25).

GENERALIZED MODULES OF MEMBRANE ANTIGENS

A high-yield production process for outer membrane particles from genetically modified bacteria was initially developed in the *Shigella* model (26). This system, denominated generalized modules of membrane antigens (GMMA), was developed in association with a facilitated two-step protocol of OMV isolation, establishing an affordable model for manufacturing bacterial EVs for use as vaccine candidates (24). This experimental system was further implemented for the analysis of nontyphoidal *Salmonella* (27), *Neisseria meningitidis* (28), *Salmonella typhimurium* (29), and, more recently, *Salmonella* Paratyphi A (10). In all cases, bacterial engineering allowed the production of tailored vesicles containing multiple agents. Indeed, OMVs can be decorated with heterologous polysaccharide or protein antigens, leading to a strong and effective antigen-specific humoral immune response in mice. Importantly, GMMA promote enhanced immunogenicity compared to traditional formulations (24).

A BIVALENT VACCINE CANDIDATE AGAINST ENTERIC FEVER

In the search for vaccines with the capacity to prevent both typhoid and paratyphoid fever, Gasperini and colleagues engineered *S. Paratyphi* A to generate GMMA displaying the heterologous *S. Typhi* Vi antigen together with the homologous O:2 antigen (10). After confirmation of the presence of both antigens at the bacterial surface, their concentration was determined in the engineered OMVs. These vesicles were used to immunize mice, which produced a strong IgG response against both the Vi and O:2 polysaccharides. Sera from these mice manifested bactericidal activity against the *S. Paratyphi* A test strain. Importantly, these sera showed antibacterial activity against a *C. freundii* strain, which displays the Vi antigen, but not any other *Salmonella*-

specific O antigen determinants (10). This established a strong proof of concept showing that both O and Vi antigens can be delivered using a vesicle-based vaccine platform to induce strong and functional antibody responses against different polysaccharides.

PERSPECTIVES: ENGINEERED EVS FOR THE PREVENTION OF INFECTIOUS DISEASES

The findings described in the study by Gasperini and colleagues (10) validate a concept that is applicable to other infectious diseases. Their study demonstrated that bacterial outer membrane vesicles represent a flexible, affordable, and highly immunogenic platform for the development of multivalent *Salmonella* vaccines. They also provided insights that might be important to other models. For instance, there are no licensed vaccines for fungal diseases (30). No human vaccines have yet been licensed for malaria or Chagas' disease, and no modern human vaccine is currently licensed against visceral or cutaneous leishmaniasis (31). The situation is similar in other models, including helminthic diseases. Vaccines are believed to offer the best chance of achieving the goal of schistosomiasis elimination, but licensed vaccines are still not available (32). All of these diseases are caused by pathogens that produce immunologically active EVs (19, 21, 22, 33–35). The use of native EVs as vaccines, however, raises many concerns, including low yields of EV collection, compositional diversity among different strains, differences in the relative concentration of immunogens with paradoxical biological activities, and the export of toxic components within the EVs (36). Such problems might be overcome in systems where the vesicles are engineered to contain major immunogens that will stimulate the immune system in favor of the host. Multivalent, EV-based vaccines also could offer broader protection. The field as a whole is still in its infancy, but the perspectives are limitless.

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