INFECTION

Outer membrane vesicle vaccines for *Neisseria gonorrhoeae*

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Multidrug-resistant *Neisseria gonorrhoeae* is a global health problem, and the development of a vaccine is considered essential for the effective control of gonorrhoea. The use of outer membrane vesicle vaccines to prevent *N. gonorrhoeae* infection has garnered considerable interest, and a recent study using a mouse model of experimental gonococcal infections adds support for this approach.

Refers to Matthias, K. A. et al. Meningococcal detoxified outer membrane vesicle vaccines enhance gonococcal clearance in a murine infection model. *J. Infect. Dis.* https://doi.org/10.1093/infdis/jiab450 (2021).

Gonorrhoea is a global public health concern, owing to its high prevalence, the severe sequelae that can result from infection, and the increasing difficulty in treating infections caused by multidrug-resistant strains of *Neisseria gonorrhoeae*[1,2]. An estimated 87 million new cases of gonorrhoea arise each year and a substantial proportion of infections remain asymptomatic, particularly genital infections in women and rectal and pharyngeal infections in men and women, and are, therefore, frequently undiagnosed and untreated[3]. If untreated, infection can lead to serious sequelae, including pelvic inflammatory disease, adverse pregnancy outcomes, neonatal complications, infertility and increased risk of HIV[3].

The incidence of gonorrhoea is increasing worldwide and is expected to continue to rise owing to increasing treatment failures caused by multidrug resistance[3]. *N. gonorrhoeae* has developed resistance to all classes of antibiotics that have been used to treat it since the 1940s, and new treatment regimens are, therefore, required every 5–10 years[3]. The current antibiotic resistance crisis and the insidious nature of gonococcal infection — whereby asymptomatic disease can lead to serious sequelae — indicate that the development of a vaccine is key for the long-term control of gonorrhoea[1]. Unfortunately, gonococcal vaccine development has been hampered by several factors including the variability and diversity of *N. gonorrhoeae* strains, which have made vaccine antigen selection problematic, the lack of a protective immune response following infection, which means that no natural correlate of protection is available to guide vaccine development, and the lack of an animal model that accurately mimics all aspects of disease and transmission (as *N. gonorrhoeae* is an obligate human pathogen), which has complicated the evaluation of vaccine candidates[2]. Despite these barriers, extensive work is underway to develop a gonococcal vaccine, with various candidate antigens under preclinical investigation[2], and several recent studies support the feasibility of outer membrane vesicle (OMV) vaccines.

Epidemiological studies have indicated that a moderate level of effectiveness against infection with *N. gonorrhoeae* might be afforded by using OMV vaccines that are licensed for use against the closely related bacteria *Neisseria meningitidis*[4–7]. OMVs are small, ~50 nM particles that are produced by many Gram-negative bacteria as outer membrane 'blebs' that present a range of surface antigens. A retrospective case–control study from New Zealand showed that individuals vaccinated with the meningococcal serogroup B OMV vaccine MeNZB were significantly less likely to contract gonorrhoea than unvaccinated controls, and MeNZB had a predicted vaccine effectiveness of 31% against *N. gonorrhoeae*[6]. A newer vaccine, 4CMenB, contains the MeNZB OMVs plus three recombinant protein antigens; antibodies raised in humans to 4CMenB are cross-reactive with *N. gonorrhoeae*[8]. These findings suggest that protective antigens exist for *N. gonorrhoeae* and a vaccine-induced immune response could lead to protection. Several studies[9] and clinical trials (such as ACTRN12619001478101, NCT04415424 and NCT04094883) are focused on engineering the OMV vaccines, identifying key antigens in the OMVs, understanding the immune response elicited to OMVs, or determining the level of potential OMV-mediated protection against *N. gonorrhoeae*.

In the recent report by Matthias et al.[10], detergent-extracted OMV (dOMV) vaccines were investigated in a murine lower genital tract infection model. These dOMVs were generated from *N. meningitidis* wild-type or mutant strains (OCh- and ΔABR) that were deleted or modified for the PorA, PorB and RmpM major outer membrane proteins (OMPs), which are variable, immunodominant and associated with evasion of the immune response. In the initial experiment, mice immunized with the OMP-deficient dOMVs, but not the wild-type dOMVs, demonstrated statistically significant enhanced gonococcal clearance compared with control mice at day 7 (56% OCh-, 77% ΔABR immunized mice cleared versus 17% Alum-immunized, 19% unimmunized mice cleared). In the second experiment, all dOMV-immunized mice had a statistically significant enhanced gonococcal clearance relative to control mice at day 10 (71–72% wild-type, OCh- and ΔABR immunized mice cleared versus 25% Alum-immunized, 33% unimmunized mice). No reproducible, significant reduction in bacterial burden was seen in dOMV-immunized mice versus Alum-immunized mice in either experiment, and sera from mice immunized with the dOMV vaccines did not induce bactericidal activity against *N. gonorrhoeae*. Antibody-dependent serum bactericidal activity correlates with protection for *N. meningitidis* in humans; however, its role in protection against *N. gonorrhoeae* is unknown. Clearance in dOMV-immunized mice was proposed to be associated with serum and vaginal IgG antibodies that cross-reacted with *N. gonorrhoeae*, but a high degree of variability in the antibody

responses was seen between the two experiments and additional research is needed to better understand the link between antibody levels and functional activity and gonococcal clearance.

Of key interest is the finding by Matthias et al.[10] that removal of PorA, PorB and RmpM from the ΔABR dOMVs resulted in generation of antibodies that recognized a wider breadth of gonococcal antigens than dOMVs from the wild-type strain, which probably explains the greater rates of clearance in ΔABR dOMV-immunized mice. Furthermore, serum from the immunized mice was used to identify cross-reactive proteins and putative vaccine targets, including PilQ, MtrE, NlpD and GuaB. This study represents a step forward towards a gonococcal vaccine; however, it also highlights the limitations and variability of the mouse model of gonococcal infection.

This work adds to growing evidence that OMV-based vaccines could provide protection against *N. gonorrhoeae* and that presentation of a wide repertoire of antigens in the form of engineered OMV vaccines is an attractive approach for vaccine development against a species as diverse and variable as *N. gonorrhoeae*.

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Competing interests
E.A.S. and K.L.S. hold patents relating to gonococcal vaccine antigens.