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A broadly-protective vaccine against meningococcal disease in sub-Saharan Africa based on Generalized Modules for Membrane Antigens (GMMA)

Oliver Koeberling[a], Emma Ispasanie[b,c], Julia Hauser[b,c], Omar Rossi[a], Gerd Pluschke[b,c], Dominique A. Caugant[d], Allan Saul[a], Calman A. MacLennan[a,e,*]

[a] Novartis Vaccines Institute for Global Health, Siena, Italy
[b] Swiss Tropical and Public Health Institute, Basel, Switzerland
[c] University of Basel, Switzerland
[d] Norwegian Institute of Public Health, Oslo, Norway
[e] University of Birmingham, Birmingham, United Kingdom

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ABSTRACT

Introduction: Neisseria meningitidis causes epidemics of meningitis in sub-Saharan Africa. These have mainly been caused by capsular group A strains, but W and X strains are increasingly contributing to the burden of disease. Therefore, an affordable vaccine that provides broad protection against meningococcal disease in sub-Saharan Africa is required.

Methods: We prepared Generalized Modules for Membrane Antigens (GMMA) from a recombinant serogroup W strain expressing PorA P1.5,2, which is predominant among African W isolates. The strain was engineered with deleted capsule locus genes, *lpxL1* and *gna33* genes and over-expressed fHbp variant 1, which is expressed by the majority of serogroup A and X isolates.

Results: We screened nine W strains with deleted capsule locus and *gna33* for high-level GMMA release. A mutant with five-fold increased GMMA release compared with the wild type was further engineered with a *lpxL1* deletion and over-expression of fHbp. GMMA from the production strain had 50-fold lower ability to stimulate IL-6 release from human PBMC and caused 1000-fold lower TLR-4 activation in Human Embryonic Kidney cells than non-detoxified GMMA. In mice, the GMMA vaccine induced bactericidal antibody responses against African W strains expressing homologous PorA and fHbp v.1 or v.2 (geometric mean titres [GMT] = 80,000–200,000), and invasive African A and X strains expressing a heterologous PorA and fHbp variant 1 (GMT = 20–2500 and 18–5500, respectively). Sera from mice immunised with GMMA without over-expressed fHbp v.1 were unable to kill the A and X strains, indicating that bactericidal antibodies against these strains are directed against fHbp.

Conclusion: A GMMA vaccine produced from a recombinant African *N. meningitidis* W strain with deleted capsule locus, *lpxL1*, *gna33* and overexpressed fHbp v.1 has potential as an affordable vaccine with broad coverage against strains from all main serogroups currently causing meningococcal meningitis in sub-Saharan Africa.

1. Introduction

Neisseria meningitidis is a major cause of epidemics in sub-Saharan Africa [1]. These were mainly caused by strains belonging to capsular group A, but there has been an increasing contribution of serogroups W and X strains with epidemic potential in the last two decades [2–5]. A serogroup A polysaccharide conjugate vaccine (MenAfriVac) has been developed for preventive mass immunization in the African meningitis belt [6]. The vaccine is highly effective at prevention of serogroup A invasive disease and carriage [7–9], but group W and X strains remain a persistent problem. This underlines the need for an affordable vaccine that provides protection against the main serogroups causing meningitis in Africa and potentially against serogroups that may emerge in the region in the future.

* Corresponding author at: Novartis Vaccines Institute for Global Health, Via Fiorentina 1, 53100 Siena, Italy. Tel.: +39 0577 539240; fax: +39 0577 243352.
E-mail addresses: calman.maclennan@novartis.com, c.maclennan@bham.ac.uk (C.A. MacLennan).

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GMMA generated from strains engineered to over-express immunogenic antigens that are present across all serogroups, constitute an attractive approach to vaccination. The term GMMA (Generalised Modules for Membrane Antigens) provides a clear distinction from conventional detergent-extracted outer membrane vesicles (dOMV), and native outer membrane vesicle (NOMV), which are released spontaneously from Gram-negative bacteria. GMMA differ in two crucial aspects from NOMV. First, to induce GMMA formation, the membrane structure has been modified by the deletion of genes encoding key structural components, including *gna33* (meningococcus) and *tolR* (*Shigella* and *Salmonella* [10]). Second, as a consequence of the genetic modification, large quantities of outer membrane bud off (the Italian word for bud is 'gemma') to provide a practical source of membrane material for vaccine production, leading to potential cost reduction. While NOMV have been used for immunogenicity studies, the yields are too low for practical vaccines.

The most promising candidate protein vaccine antigen discovered for meningococcus is factor H binding protein fHbp. The extraction process required to make dOMV removes lipoproteins, including fHbp, and increases the cost of production of dOMV relative to GMMA. The fHbp gene is present in most invasive meningococcal isolates independent of the serogroup. fHbp can be divided into three antigenic variants (v. 1, 2 or 3) [11] or into at least nine modular groups based on the combination of five variable α and β fHbp segments [12,13]. Individual peptides within each variant are identified by a unique peptide ID. The outer membrane protein, PorA, is highly immunogenic but antibodies tend to provide subtype-specific protection [14]. African meningococcal isolates are relatively conserved in relation to fHbp variant and PorA subtype [15,16]. Invasive serogroup A and X strains predominantly express fHbp v.1. PorA subtype P1.5,2 is shared by most serogroup W strains and P1.20,9 is expressed by the majority of A strains [15]. This epidemiological pattern makes a protein-based vaccine both a possible and attractive approach for sub-Saharan Africa.

A vaccine for the meningitis belt needs to be affordable and large-scale low-cost production of a GMMA vaccine has to be feasible. Deletions of *gna33* or *rmpM*, that augment the release of these outer membrane particles can reduce costs [17–21]. In this study, we selected a vaccine strain based on a panel of African W strain capsule and *gna33* double knock-out mutants. The isolate with the highest GMMA production was then further engineered for the deletion of *lpxL1* and over-expression of fHbp v.1 (ID1). This genetic approach may form the basis for a broadly-protective, safe and economic vaccine for sub-Saharan Africa.

2. Materials and methods

2.1. N. meningitidis strains

Three African serogroup W, seven A and seven X strains were the target strains for serum bactericidal assays. Nine African serogroup W strains were screened as potential vaccine production strains (Table 1). Carrier strain 1630 (ST-11) expressing PorA subvariant P1.5,2 and fHbp v.2 (ID23) was chosen for GMMA production [22]. To abolish capsule production, a fragment of the bacterial chromosome containing *synX*, *ctrA* and the promoter controlling their expression, was replaced with a spectinomycin-resistance gene. First, the recombination sites were amplified with primers ctrAf_Xma:CCCCCCGGGCAGGAAAGCGCTGCATAG and ctrAr_XbaCGTCTAGAGGTTCAACGGCAAATGTGC; Synf_KpnCGGGGTACCCGTGGAATGTTTCTGCTCAA and Synr_SpeGGACTAGTCCATTAGGCCTAAATGCCTG from genomic DNA from strain 1630. The fragments were inserted into plasmid pComPtac [23] upstream and downstream of the chloramphenicol resistance gene. Subsequently the chloramphenicol resistance gene was replaced with a spectinomycin resistance cassette. The *lpxL1* gene was deleted by replacement with a kanamycin resistance gene [24], and the *gna33* gene with an erythromycin resistance cassette [25]. fHbp expression was up-regulated using multicopy plasmid encoding fHbp v.1 (ID1) [26].

2.2. GMMA preparation

Bacteria were grown at 37 °C, 5% CO_2 in 50 mL of a modified version of a meningococcus defined medium described previously [27] at 180 rpm until early stationary phase. Cells were harvested (2200 g, 30 min, 4 °C) and the culture supernatant containing the GMMA was filtered through a 0.22 μm pore-size membrane (Millipore, Billerica, MA, USA). To collect GMMA, the supernatant was ultracentrifuged (142,000 × *g*, 2 h, 4 °C). The membrane pellet was washed with phosphate buffered saline (PBS), resuspended in PBS and sterile filtered. GMMA concentration was measured according to protein content by Lowry assay (Sigma–Aldrich, St. Louis, MO, USA). For protein and lipooligosaccharide analysis, GMMA were separated by SDS–PAGE using a 12% gel and MOPS or MES buffer (Invitrogen, Carlsbad, CA, USA). Total proteins were stained with Coomassie Blue stain. The amount of PorA was determined by densitometric quantification of the PorA protein in relation to total measurable protein. Lipooligosaccharide was visualized by treatment of the gel with periodic acid and staining with silver nitrate. The gel was developed with a solution containing 50 mg/L citric acid and 0.05% formaldehyde. fHbp was detected by Western blot using a polyclonal antibody raised in mice against recombinant fHbp ID1.

2.3. IL-6 release by human peripheral blood mononuclear cells (PBMC) stimulated with GMMA

PBMC were separated from whole blood using Ficoll-Paque Plus density gradient (Amersham Pharmacia Biotec), washed with PBS and resuspended in 10% heat-inactivated fetal bovine serum (FBS)/10% Dimethyl sulfoxide and stored in liquid nitrogen until use. For stimulation, PBMCs were thawed, washed with PBS/2.5 mM EDTA and 20 μg/mL DNAse (Sigma–Aldrich, St. Louis, MO, USA) and resuspended in RPMI-1640 complete (with 25 mM HEPES, glutamine, 10% FBS + 1% Antibiotics Pen-Strep). 2×10^5 cells/well were stimulated with GMMA (1–10^{-6} μg/mL final concentration) for 4 h at 37 °C. Cells were removed by centrifugation and IL-6 in the supernatants was measured by ELISA using 0.1 μg of an anti-human IL-6 antibody (eBioscience, San Diego, CA, USA). A Biotin-labelled anti-human IL-6 antibody was used for detection (e-Bioscience).

2.4. Measurement of TLR-4 stimulation by NF-κB luciferase reporter assay

Human Embryonic Kidney 293 (HEK293) cells expressing luciferase under control of the NF-κB promoter and stably transfected with human Toll-like receptor (TLR) 4, MD2 and CD14 were used. 25,000 cells/well were added to microclear luciferase plates (PBI International) and incubated for 24 h at 37 °C. GMMA (1–1.28×10^{-5} μg/mL final concentration) were added and incubated for 5 h. Cells were separated from the supernatant and lysed with passive lysis buffer (Promega, Madison, WI, USA). Luciferase assay reagent (Promega) was added and fluorescence was detected using a luminometer LMaxII 384 (Molecular Devices).

2.5. Mouse immunization

Female CD-1 mice were obtained from Charles River Laboratories (Wilmington, MA, USA). Eight mice per group were immunised