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Immuno-proteomic analysis of human immune responses to experimental *Neisseria meningitidis* outer membrane vesicle vaccines identifies potential cross-reactive antigens

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ABSTRACT

Human volunteers were vaccinated with experimental *Neisseria meningitidis* serogroup B vaccines based on strain H44/76 detoxified L3 lipooligosaccharide (LOS)-derived outer membrane vesicles (OMV) or the licensed Cuban vaccine, VA-MENGOC-BC. Some volunteers were able to elicit cross-bactericidal antibodies against heterologous L2-LOS strain (760676). An immuno-proteomic approach was used to identify potential targets of these cross-bactericidal antibodies using an L2-LOS derived OMV preparation. A total of nine immuno-reactive spots were detected in this proteome: individuals vaccinated with the detoxified OMVs showed an increase in post-vaccination serum reactivity with Spots 2–8, but not with Spots 1 and 9. Vaccination with VA-MENGOC-BC induced sera that showed increased reactivity with all of the protein spots. Vaccinees showed increases in serum bactericidal activity (SBA) against the heterologous L2-LOS expressing strain 760676, which correlated, in general, with immunoblot reactivity. The identities of proteins within the immuno-reactive spots were determined. These included not only well-studied antigens such as Rmp, Opa, PorB and FbpA (NMB0634), but also identified novel antigens such as exopolyphosphatase (NMB1467) and γ-glutamyltranspeptidase (NMB1057) enzymes and a putative cell binding factor (NMB0345) protein. Investigating the biological properties of such novel antigens may provide candidates for the development of second generation meningococcal vaccines.

1. Introduction

Neisseria meningitidis (meningococcus) infections are still of considerable concern worldwide, leading to significant mortality and morbidity [1]. Capsular polysaccharide (CPS)-conjugate vaccines have proven success in virtually eliminating disease caused by serogroup C meningococci and serogroup A, Y and W CPS-conjugate vaccines are expected to deliver a similar outcome in those countries where they are introduced [2–4]. The development of meningococcal serogroup B (MenB) vaccines has proved more intractable, due to the poor immunogenicity of the B capsule and its molecular mimicry of human fetal NCAM that raises concerns over inducing auto-immune responses [5]. Studies on the sub-capsular outer membrane (OM) have led to the development and use of OM vesicle (OMV) vaccines, which are based on detergent extraction of OM to reduce lipopolysaccharide content and reactogenicity [6]. Such vaccines have been successful in controlling clonal outbreaks of MenB disease, e.g. in Cuba [7], Brazil [8] and more recently in New Zealand, through the use of a strain-specific vaccine MeNZB (NZ98/254, P1.7–2,4, ST41/44) [9].

MeNZB is also a component of Bexsero®, which has been recently licensed as a MenB vaccine by the European Union [10,11]. Inclusion of MenZB is believed to provide an immuno-adjuvant effect for the defined recombinant antigens – Neisseria heparin binding antigen (NHBA, genome-derived Neisseria antigen (GNA) 2132), factor H binding protein (fHbp or lipoprotein (LP)2086, GNA1870), and Neisseria adhesin A (NadA, GNA1994) [12] – and this adjuvant effect may be related to the lipid vesicular structure itself and the presence of immunomodulatory OM antigens. MeNZB is included also to provide additional protection against ST41/44 clonal complex strains, particularly since low level expression of fHbp protein on the surfaces of meningococcal strains of clonal complex 41/44 has been reported [13–15], suggesting the possibility that such strains may be more difficult to kill with a vaccine response towards one of the recombinant proteins within

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Bexsero®. The proteome of the meningococcal OM/OMV is complex, containing >200 proteins [16–20] and the hypervariable PorA protein is the immunodominant antigen that generates serum bactericidal antibodies, the generally accepted correlate of protection [21]. It is also possible that immunomodulatory components of the OM/OMV synergistically contribute to the stimulation of bactericidal antibodies.

In the current study, we used an immuno-proteomics approach to investigate pre- and post-vaccination sera from human volunteers vaccinated with experimental meningococcal serogroup B vaccines [22]. The trial assessed the immunogenicity of five formulations of detoxified L3 derived lipooligosaccharide OMV (TrL3 or L7 OMV vaccines) produced in strain H44/76 background alongside the licensed Cuban VA-MENGOC-BC vaccine [7]. The modified LOS vaccines lacked PorA and tended to be less immunogenic than VA-MENGOC-BC, which contains PorA (P1.15,19). Regardless, paired sera were identified from individuals vaccinated with TrL3 or L7 OMV that showed significant increases in post-vaccination serum bactericidal activity (SBA), which could be used in the immuno-proteomic study. The trial also highlighted that the response of individuals to VA-MENGOC-BC, which expresses the L3 LOS immunotype, was high towards a heterologous L2 LOS strain (760676). Since no cross-reactivity has been observed between the L2 and L3 immunotypes [23], the authors suggested that the cross-protective responses towards the L2 strain could be directed against as yet uncharacterized minor OM proteins. Thus, our immuno-proteomic study relates increases in serum reactivity with the proteome of the heterologous L2-LOS derived OMV with increases in post-vaccination SBA against the corresponding L2 strain, in order to potentially identify those minor OM antigens with cross-reactivity.

2. Materials and methods

2.1. OMV vaccines and serum samples

A total of 26 paired pre- and post-vaccination serum samples were available from young adults vaccinated in an open, randomized phase I trial with experimental *N. meningitidis* serogroup B (MenB) vaccines obtained from strain H44/76 (B:15:P1.7,16:L3,7,9 ST32 complex) detoxified L3 derived lipooligosaccharide (LOS) OMV (TrL3 or L7 OMV): specifically, 8 paired samples were from subjects receiving TrL3 OMVs and 18 paired samples from subjects receiving L7 OMVs [22]. An additional 9 paired sera were from individuals vaccinated with the VA-MENGO-BC vaccine (Finlay Institute, Cuba) [7]. Subjects were selected based on their pre-immunization versus post-immunization dose III SBA titers and selecting for the highest vaccine responders.

The experimental TrL3 and L7 OMV vaccines differ only in the size of the LOS α-chain and they were obtained by inactivation of the *lst* and *lgtB* genes, respectively [22,24]. They also do not contain either PorA or FrpB protein, which were both removed by gene-deletion. The rationale for PorA and FrpB deletion was to improve the immunogenicity of minor OM proteins, including Hsf (also known as NhhA, neisserial autotransporter) and Tbp (transferrin binding protein)A, which were over-expressed in the OM by genetic manipulation and growth under iron depletion, respectively [22]. The relative content of Hsf and TbpA were estimated against PorB content using SDS-PAGE and were found to be present in the OM at ~5% and ~15% of the PorB content, respectively [22]. In addition, data from a previous study [23] have shown that in the absence of up-regulation and using classical culture, i.e. no iron chelation, the level of Hsf and TbpA were so low that they could not be detected by SDS-PAGE. Based on one-dimensional SDS-PAGE, the expression of other antigens in these OMV vaccines was identical, as previously reported [22,24].

The VA-MENGOC-BC vaccine is not genetically modified and contains wild-type OMVs of serogroup B strain CU385 (B:4:P1.19,15; L3,7,9 ST32 complex) produced by a detergent extraction method, in combination with serogroup C polysaccharide [7].

2.2. Heterologous OMV preparation for immuno-proteomics

A heterologous L2-LOS OMV preparation was made from *N. meningitidis strain* 760676 (B:2a:P1.5,2:L2 ST11 complex) by sodium deoxycholate (NaDOC, 0.1% w/v) extraction, as described previously [22]. The strain was genetically modified [22] to produce blebs that lacked PorA and capsule and contained a non-sialylated LOS, thanks, respectively, to *porA*, *siaD*, *msbB* and *lst* gene deletion. The strain was also grown in the presence of iron (to avoid expression of iron binding proteins like FrpB and TbpA); the rationale for this was to concentrate the immuno-proteomic search on the probably minor cross-reactive, and possibly more conserved, antigens without focusing on the responses to the more variable iron-regulated proteins. The choice of L2-LOS derived heterologous OMV was also made to reduce the possibility of having immune-reactive spots linked to a LOS response and thus to focus on the anti-protein response.

2.3. Serum bactericidal activity (SBA)

Bactericidal activity of pre- and post-vaccination sera was determined against the homologous H44/76 and heterologous 760676 strains using the GlaxoSmithKline Vaccines Laboratory standard SBA-MenB assay with human complement, as described previously [25].

2.4. 2-Dimensional (2D) gel electrophoresis and western blot assay

L2-LOS OMV samples were subjected to 2D gel electrophoresis in triplicate as described previously [26]. For each serum sample examined, one 2D gel (reference gel) was stained with ProteoSilver plus staining kit (Sigma) to visualize proteins. The two unstained 2-D gels were electroblotted onto polyvinylidene difluoride membranes, stained with MemCode protein stain (Perbio Science) and the blots scanned to produce a reference map of proteins. The membrane stain was then reversed, following the manufacturer's instructions, and the membranes incubated with individual serum (1/300 dilution) samples and immunoreactivity detected as described previously [26]. Membranes were then scanned, and the profile of immuno-reactive proteins matched to 2D gel images of the same sample using PDQuest software (Bio-Rad). The signal intensities of individual antigen reactions were compared and scored semi-quantitatively by three independent investigators on a scale of 0 to 5, as described previously [26]. The profiles of immuno-reactive proteins were matched to 2D gel images of the same sample and selected protein spots were excised from the stained 2D gels, digested in-situ with trypsin [27] and subjected to mass spectrometry fingerprinting.

2.5. Mass spectrometry (MS) and data processing

LC–MS–MS was done as described previously [20] and MS–MS data were searched against a protein translation of both the MC58 genome and the NCBI non-redundant database in a FASTA format using MASCOT (Matrix Science, London, U.K.). The significance