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The Serogroup B Meningococcal Vaccine Bexsero Elicits Antibodies to *Neisseria gonorrhoeae*

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Background. *Neisseria gonorrhoeae* and *Neisseria meningitidis* are closely-related bacteria that cause a significant global burden of disease. Control of gonorrhoea is becoming increasingly difficult, due to widespread antibiotic resistance. While vaccines are routinely used for *N. meningitidis*, no vaccine is available for *N. gonorrhoeae*. Recently, the outer membrane vesicle (OMV) meningococcal B vaccine, MeNZB, was reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand. To probe the basis for this protection, we assessed the cross-reactivity to *N. gonorrhoeae* of serum raised to the meningococcal vaccine Bexsero, which contains the MeNZB OMV component plus 3 recombinant antigens (Neisseria adhesin A, factor H binding protein [fHbp]-GNA2091, and Neisserial heparin binding antigen [NHBA]-GNA1030).

Methods. A bioinformatic analysis was performed to assess the similarity of MeNZB OMV and Bexsero antigens to gonococcal proteins. Rabbits were immunized with the OMV component or the 3 recombinant antigens of Bexsero, and Western blots and enzyme-linked immunosorbent assays were used to assess the generation of antibodies recognizing *N. gonorrhoeae.* Serum from humans immunized with Bexsero was investigated to assess the nature of the anti-gonococcal response.

Results. There is a high level of sequence identity between MeNZB OMV and Bexsero OMV antigens, and between the antigens and gonococcal proteins. NHBA is the only Bexsero recombinant antigen that is conserved and surfaced exposed in *N. gonorrhoeae*. Bexsero induces antibodies in humans that recognize gonococcal proteins.

Conclusions. The anti-gonococcal antibodies induced by MeNZB-like OMV proteins could explain the previously-seen decrease in gonorrhoea following MeNZB vaccination. The high level of human anti-gonococcal NHBA antibodies generated by Bexsero vaccination may provide additional cross-protection against gonorrhoea.

Keywords. STI; gonorrhea; *Neisseria gonorrhoeae*; immune response; meningococcal vaccine.

The sexually transmitted infection gonorrhoea is a global public health concern [1]. It is estimated that there are approximately 100 million cases of gonorrhoea worldwide each year [2], with the number of cases rising in recent years [3, 4]. Symptomatic gonococcal infections most commonly present as urethritis in males and cervicitis in females, although mucosal infections of the rectum, pharynx, and eye frequently occur. Furthermore, asymptomatic infections are common and, if undiagnosed or untreated, gonorrhoea can lead to severe sequelae, including pelvic inflammatory disease, adverse pregnancy outcomes, neonatal complications, and infertility. Gonococcal infection also increases the risk of human immunodeficiency virus [1, 5]. The effectiveness of antibiotics has been significantly compromised, and strains with high-level resistances to the last line of antibiotics, the expanded-spectrum cephalosporins, have been isolated from around the world [6]. As such, *N. gonorrhoeae* has been prioritized as an urgent public health threat for which immediate action is needed [7, 8], including the development of a gonococcal vaccine [9].

Vaccine development has been challenging for *N. gonorrhoeae*, and none of the vaccine candidates tested in clinical trials have afforded protection against gonorrhoea. This is largely due to its various mechanisms of immune evasion, the lack of an animal model that mimics natural disease, and our limited understanding of what is required to induce a protective immune response [1, 10]. However, several different approaches have identified promising gonococcal vaccine candidates [11–17]. In addition, recently, a vaccine to a closely-related pathogen, the *Neisseria meningitidis* serogroup B outer membrane vesicle (OMV) vaccine MeNZB, was associated with decreased rates of gonorrhoea [18, 19]. MeNZB was developed in response to a meningococcal epidemic in New Zealand, and over 1 million people were vaccinated between 2004 and 2008 [20]. A retrospective case-control study showed that individuals vaccinated with MeNZB were significantly less likely to contract

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gonorrhoea compared with unvaccinated controls, with a predicted vaccine efficacy of 31% [18].

OMVs are spherical, bi-layered membrane structures that are naturally released from the outer membrane of Gram-negative bacteria and contain phospholipids, lipopolysaccharides (LPS), and a mix of outer membrane proteins [21, 22]. The most abundant proteins in meningococcal OMVs include PorA, PorB, and OpcA, with the antigenically-diverse PorA being immunodominant and the main target of serum bactericidal antibodies [23]. However, functional antibodies are raised against other OMV components, and some cross-protection against heterologous strains with mismatched PorA types has been reported [24]. Despite causing distinct diseases, *N. meningitidis* and *N. gonorrhoeae* are genetically and antigenically very similar, with 80–90% nucleotide identity across the genome and many proteins sharing high levels of identity (eg, PorB shares 60–70% amino acid homology) [14, 25]. As such, meningococcal OMV vaccines may induce functional antibodies against gonococcal strains. Several other observational studies have also reported reduced rates of gonorrhoea following the use of OMV-based meningococcal vaccines [26–30].

MeNZB is no longer available. However, the broad-spectrum serogroup B vaccine Bexsero contains the MeNZB OMV antigen plus 3 recombinant antigens (Neisseria adhesin A [NadA], factor H binding protein [fHbp]-GNA2091, and Neisserial heparin binding antigen [NHBA]-GNA1030) [31]. NadA, fHbp, and NHBA induce serum bactericidal antibodies against diverse strains [32, 33]. The accessory proteins GNA2091 [34] and GNA1030 [35] are fused with fHbp and NHBA, respectively, and increase their immunogenicity and serum bactericidal titers [33]. In *N. gonorrhoeae,* the gene encoding NadA is absent [36, 37]; the gene encoding fHbp is present, but not surface exposed [38]; and genes encoding NHBA, GNA2091, and GNA1030 are present [36, 37], but have not been characterized in detail. NHBA was found to be present in 17/17 *N. gonorrhoeae* strains studied, with an average identity of 81.2% to NHBA-2 peptide in Bexsero [37], and in 97/111 strains, *N. gonorrhoeae* had a 65.6% identity to the non-vaccine NHBA-3 peptide from *N. meningitidis* strain MC58 [36]. Here, we investigated the similarity of antigens present in MeNZB and Bexsero to gonococcal proteins, the capacity of MeNZB-like OMVs and Bexsero recombinant antigens to induce anti-gonococcal antibodies, and the specificity of antibodies induced by Bexsero-vaccinated humans to recognize gonococcal surface antigens.

METHODS

Bacterial Strains

N. gonorrhoeae strains 1291, FA1090, and WHO K were grown at 37°C with 5% CO_2 on GC agar or broth (Oxoid) supplemented with IsoVitalex (Becton Dickinson).

Sequence Analysis

Allele and protein sequences of vaccine antigens are shown in Table 1. Sequences were aligned with CLUSTAL in MEGA7, and the percentage of amino acid identity and similarity were calculated (BLOSUM90, threshold 0). The protein identity between gonococcal strains was determined using the Basic Local Alignment Search Tool program (BLASTp) with sequences from *N. gonorrhoeae* 1291 against 438 gonococcal genomes in GenBank.

NHBA distribution was investigated in >3000 *N. gonorrhoeae* genomes (PubMLST [39]) using BLASTx with NHBA from *N. gonorrhoeae* 1291 (GenBank Accession EEH61857.1). Amino acid sequence alignments, phylogeny tree construction, and annotation were performed using Clustal Omega at EMBL-EBI, MEGA (v7.0.26), and iTOL (v3.5.4), respectively.

Outer Membrane Vesicle Preparation

Naturally-secreted gonococcal OMVs were isolated as described previously [40]. Briefly, OMVs were harvested from a 6-hour culture (OD_{600} ~0.8) by brief centrifugation (5000 x *g*), the supernatant was filtered (0.22μm filter), the filtrate was centrifuged (100 000 x *g*, 1 hour, 4°C), the pellet was washed with phosphate buffered saline (PBS), then OMVs were solubilized in PBS-0.2% sodium dodecyl sulfate (SDS).

Expression of Recombinant Neisserial Heparin Binding Antigen

Escherichia coli BL21(DE3) was transformed with pET19b carrying the mature NHBA (no signal sequence) from *N. gonorrhoeae* 1291 (amplified using 5'-ATTActcgagTCGCCCGATGTCAAGTC-3' and 5'-TGAAggatccCGGCATCAACATCAATC-3' primers containing XhoI and BamHI sites shown in lower case, in the respective primers). The expression of recombinant NHBA (rNHBA) was induced (100 mM isopropyl β-D-1-thiogalactopyranoside [IPTC], 16 hours, 25°C) and protein-purified using TALON affinity resin (Clontech), as described previously [40].

Polyclonal Rabbit Serum

The rabbit sera to Bexsero vaccine antigens were generated as per Giuliani et al [33] and were provided by Novartis Vaccines. On days 0, 21, and 35, New Zealand White rabbits were immunized with 10 μg NZ98/254 OMV (α-OMV) or a combination of 25 μg each of the recombinant antigens NadA, fHbp-GNA2091, and NHBA-GNA1030 (α-rMenB) with aluminum hydroxide. Blood was taken on day 49.

Human Serum

Pre- and post-vaccination human serum was obtained from a previous phase II trial, in which adult laboratory staff were vaccinated with 3 doses of Bexsero at 0, 3, and 6 months [41]. Pre-vaccination (month 0) and 1 month post–dose 3 of Bexsero (month 7) serum samples from 10 individuals were tested. In addition, samples from healthy adults with no history of meningococcal disease who were vaccinated

Table 1. Bexsero Vaccine Components and Their Homology to Gonococcal Proteins

OMV Protein Antigens				
NMB Locus[a]	Protein	Abundance in OMVs[b]	%ID to Ng FA1090[c]	%ID Between Ng Strains[d]
NMB2039[e]	PorB (porin, major OMP PIB)	42.54	67.3	88.6–100
NMB1429[e]	PorA (porin, serosubtype P1.4)	28.63	n/a	n/a
NMB1497	TonB-dependent receptor	4.60	96.1	98–100
NMB0382[e]	RmpM (OMP class 4)	3.08	93.4	99.6–100
NMB0964	TonB-dependent receptor	2.87	96.9	96.2–100
NMB1812	PilQ (Tfp assembly protein)	1.44	91.4	79.1–100
NMB0634[e]	FbpA (iron ABC transporter substrate-binding protein)	1.29	99.1	99.1–100
NMB1126/NMB1164	Putative lipoprotein NMB1126/1164	1.06	94.2	99.1–100
NMB1988[e]	FrpB (FetA, iron-regulated OMP)	0.96	94.3	94.6–100
NMB0461	Tbp1 (transferrin binding protein 1)	0.92	93.7	38.3–100
NMB0182[e]	OMP85	0.87	95	99.2–100
NMB1053[e]	OpcA (class 5 OMP)	0.75	43.8	98.9–100
NMB0088	OMP P1	0.54	94	98.9–100
NMB1540	LbpA (lactoferrin binding protein A)	0.46	n/a[f]	41.0–100
NMB0280	LptD (LPS assembly protein/organic solvent tolerance protein [OstA])	0.44	89.8	99.4–100
NMB1714	MtrE (outer membrane efflux protein)	0.29	96.4	95.3–100
NMB0109	LysM peptidoglycan-binding domain containing protein	0.26	88.7	97.3–100
NMB1333	hypothetical protein	0.24	96.3	97.3–100
NMB1567	FkpA (macrophage infectivity protein)	0.23	97.8	98.9–100
NMB0946	antioxidation AhpC TSA family glutaredoxin	0.20	98.5	99.6–100
NMB0375	MafA adhesin (mafA-1)	0.18	98.8	59.2–100
NMB0633[e]	NspA (OMP)	n/a	93.7	25.4–100

Recombinant Protein Antigens				
NMB Locus[a]	Protein	Variant (strain)[g]	%ID to Ng[c]	%ID Between Ng Strains[d]
NMB2132	Neisseria heparin binding antigen (NHBA)	peptide 2 (NZ98/254)	68.8	93.7–100
NMB1870	Factor H binding protein (fHbp)	peptide 8, variant 1.1 (MC58)	62.6[h]	98.9–100
NMB1994	Neisseria Adhesin A (NadA)	peptide 8, variant 2/3 (2996)	n/a	n/a
NMB1030	GNA1030 (NUbp)	n/a (2996)	92.6	98.8–100
NMB2091	GNA2091	n/a (2996)	95.6	99.5–100

Abbreviations: %ID, percent identity; ABC, ATP-binding cassette; AhpC, alkyl hydroperoxide reductase C; FbpA, ferric binding protein A; fHbp, factor H binding protein; FkpA, FKBP-type peptidyl-prolyl cis-trans isomerase; FrpB, Fe-regulated protein B; LbpA, lactoferrin binding protein A; LPS, lipopolysaccharides; LptD, LPS-assembly protein; LysM, Lysin Motif; MafA, multiple adhesin family A; MeNZB, OMV meningococcal B vaccine; MtrE, outer membrane efflux protein; n/a, not available; NadA, Neisseria adhesin A; Ng, *Neisseria gonorrhoeae*; NHBA, Neisserial heparin binding antigen; NMB, *Neisseria meningitidis* strain MC58; NspA, *Neisseria gonorrhoeae* surface protein A; OMP, outer membrane protein; OMV, outer membrane vesicle; OpcA, opacity protein A; PilQ, pili associated protein Q; PorA, porin, serosubtype P1.4; PorB, porin, major OMP PIB; RmpM, reduction modifiable protein M; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tbp1, transferrin binding protein 1; Tfp, type IV pili; TSA, thiol specific antioxidant.

[a]For the distribution and homology analysis of OMV proteins, allele and protein sequences were obtained from strains *Neisseria meningitidis* NZ98/254 (isolate id 34532) from the PubMLST database (https://pubmlst.org/neisseria) [39]. When the sequence for NZ98/254 was not available, the sequence from the *N. meningitidis* NZ05/33 (isolate id 19263) was used. NMB locus tags corresponding to *N. meningitidis* strain MC58 (accession NC_003112) are used as this genome is fully annotated.

[b]Average abundance, calculated from average of 6 lots of Bexsero from Table 2 in Tani et al [44]. NspA protein is detected poorly by the proteomic approached used, compared with its abundance on SDS-PAGE [44].

[c]Sequence from *Neisseria meningitidis* strain NZ05/33 (NZ98/254 genome is not available) was compared with Ng strain FA1090.

[d]Conservation of antigen in the 438 Ng genomes in GenBank.

[e]An antibody response is induced to this protein post–MeNZB vaccination [45].

[f]The gene encoding LbpA is a pseudogene in FA1090 but is expressed by the majority of gonococcal strains.

[g]Previously established nomenclature for Bexsero NHBA, fHbp, and NadA was used, where every unique peptide sequence is assigned a unique identification number (eg, NHBA peptide 2 [NHBA-2] is in Bexsero). Gray shading indicates the level of identity: dark >90%; medium >80%; light >60%.

[h]The gonococcal fHbp is not expressed on the surface of the gonococcus due to the absence of a signal sequence for export [38]. It has previously been shown that the gonococcal fHbp signal sequence differs from that of *Neisseria meningitidis* and is identical in 111 gonococcal isolates examined [36]. We confirm that the N-terminal 33 amino acids are identical in all annotated fHbp sequences in the gonococcal genome strains available in GenBank.

with 2 doses of Bexsero at 0 and 2 months (as per Australian recommendations [42]) were collected in accordance with the guidelines of the Griffith University Human Ethics Committee (HREC 2012/798).

Western Blot

Western blot analysis [40] was performed with whole-cell lysates, OMV, or rNHBA, that were separated using polyacrylamide gel electrophoresis (PAGE) with 12% Bis-Tris NuPAGE

Table 2. Enzyme-linked Immunosorbent Assay Geometric Mean Titers Against Bexsero Vaccine Components in Serum From Bexsero-vaccinated Humans

	Pre-vaccination	1 Month Post–dose 3[b]	
Antigen[a]	GMT (95% CI)	GMT (95% CI)	*P* Value
Ng OMV	34 297 (20 946–56 156)	42 224 (29 853–59 722)	.596
Ng whole cell	48 503 (25 906–90 811)	78 793 (49 228–126 115)	.035
Nm whole cell	97 006 (42 879–219 456)	388 023 (183 938–818 550)	.0091
Ng rNHBA	34 297 (20 946–56 156)	1 176 267 (669 930–2 065 300)	.0051

GMT is the arithmetic mean of the logarithms of individuals' serum titers. *P* values were calculated using the Wilcoxon signed-rank test.

Abbreviations: CI, confidence interval; GMT, geometric mean titers; Ng, *Neisseria gonorrhoeae*; Nm, *Neisseria meningitidis*; OMV, outer membrane vesicles; rNHBA, recombinant Neisseria heparin binding antigen.

[a]OMV, intact, whole cells from Ng, whole cells from Nm, or rNHBA from Ng strain 1291.

[b]From a 3-dose vaccine schedule (0, 1, and 3 months) and 10 donors.

gels. A rabbit (1:2000) or human (1:4000) primary antibody and a horseradish peroxidase (HRP)–conjugated anti-immunoglobulin secondary antibody (Sigma-Aldrich) were used for protein detection. Duplicate gels were Coomassie stained to confirm equal sample loading.

Enzyme-linked Immunosorbent Assays

Enzyme-linked immunosorbent assays (ELISAs) [40] were performed with 96-well MaxiSorp (NUNC) plates coated with *N. gonorrhoeae* or *N. meningitidis* (50 µL/well of OD_{600} 0.2), OMVs (2 µg/mL), rNHBA (1 µg/mL), or LPS (1 µg/mL [43]). Binding by rabbit or human antibodies was detected using Goat Anti-Rabbit HRP (1:2000; Dako) or Goat Anti-Human immunoglobin G Fc HRP (1:20 000; ThermoFisher), respectively. The ELISA titer is the highest serum dilution with absorbance at 450 nm > mean negative (all reagents excluding primary sera) + 3 standard deviations.

RESULTS

Gonococcal Proteins Share a High Level of Identity With Serogroup B Meningococcal Vaccine Antigens

To investigate the sequence conservation between serogroup B meningococcal vaccine antigens and *N. gonorrhoeae*, the major OMV protein antigens present in MeNZB and Bexsero and the recombinant protein antigens present in Bexsero were compared to gonococcal proteins from available *N. gonorrhoeae* genomes. OMVs contain a heterogeneous mix of numerous proteins; however, proteomic analysis of OMVs from Bexsero vaccine preparations identified a core set of 22 proteins that comprise >90% of OMV content [44]. Several of these major OMV proteins induce an antibody response to meningococcal strains post–MeNZB vaccination [45]. Our bioinformatic analysis identified homologues of 20 of the 22 core OMV proteins in *N. gonorrhoeae* strain FA1090 (Table 1). Of these 20 homologues, 16 proteins have >90% identity, 2 proteins have >80%, and 2 proteins are poorly conserved in FA1090 (PorB [24] and OpcA [46]). For the 2 proteins absent in FA1090, *porA* is a pseudogene in *N. gonorrhoeae* [47] and *lbpA* is a pseudogene in *N. gonorrhoeae* strain FA1090, but is expressed by the majority of gonococcal strains. Of the major OMV proteins that have a homologue in *N. gonorrhoeae,* 14 of these also have a high level of sequence identity (94–100%) in the 438 gonococcal genome strains available in GenBank (Table 1).

An analysis of the recombinant Bexsero antigens confirmed previous findings from smaller strain panels [36, 37] that the gene encoding NadA is absent in *N. gonorrhoeae*, while homologues of fHbp, NHBA, GNA2091, and GNA1030 are conserved *N. gonorrhoeae* strains (Table 1). Since fHbp [38], GNA2091 [34], and GNA1030 [35] are not believed to be surface-exposed in *N. gonorrhoeae*, further investigation focused on NHBA. Bexsero contains NHBA-2, which shares 68.8% identity to the NHBA variant from strain FA0190 (NHBA-527; Table 1). An investigation of NHBA in *N. gonorrhoeae* genome strains in GenBank revealed that *nhba* is present in 100% of strains. An expanded search in the PubMLST database indicated that 72% of gonococcal strains (3068/4953) have an annotated *nhba* gene (NEIS2109). This is likely an underestimate of the presence of *nhba,* due to duplicate and incompletely-annotated genomes. There are 41 unique NHBA variants reported for *N. gonorrhoeae* and 393 for *N. meningitidis* in PubMLST. There are 3 NHBA variants, represented by *N. gonorrhoeae* strains WHO K (NHBA-475), PID332 (NHBA-481), and 1291 (NHBA-542), that account for 82% of gonococcal strains, and these variants each share 67% identity to the NHBA-2 in Bexsero (Figure 1A). The phylogenetic relatedness of the most common NHBA variants (ie, variants present in ≥1% of *N. meningitidis* or *N. gonorrhoeae* strains in the database) is shown in Figure 1B. This tree highlights the relatively high conservation of NHBA in *N. gonorrhoeae*, with

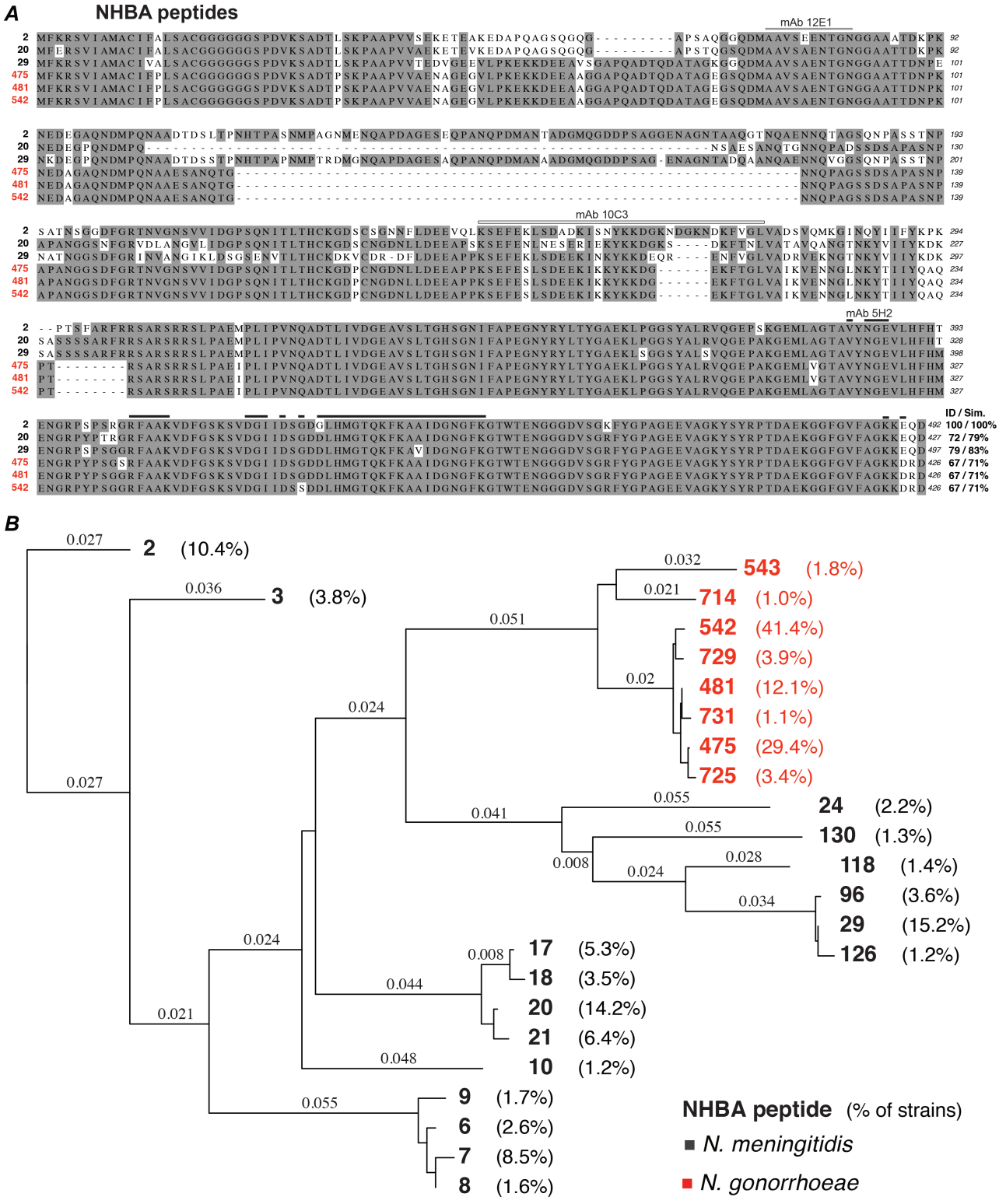

Figure 1. Conservation of Neisserial heparin binding antigen (NHBA) in *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *A*, Alignment of the amino acid sequence of the 3 main NHBA peptide variants in *N. meningitidis* (black) and *N. gonorrhoeae* (red). Bexsero contains NHBA peptide 2 from *N. meningitidis* strain NZ98/254. The percent identity and similarity of each NHBA peptide to the Bexsero NHBA peptide 2 is shown on the left. Amino acids identified in epitopes bound by the human monoclonal antibodies 12E1 (gray bar; 9/10 amino acids identical to gonococcal NHBA) and 10C3 (open bar; 24/32 amino acids identical to gonococcal NHBA) in the N-terminal region [48], and 5H2 (black bar, 32/35 amino acids identical to gonococcal NHBA) interacts with in the C-terminal of NHBA-2 [49] are indicated above the sequence. *B*, Phylogenetic tree of the most common NHBA peptides present in *N. meningitidis* (black) and *N. gonorrhoeae* (red); the percent of strains that express this peptide are shown (all peptides present in ≥1% of strains are shown). The Bexsero NHBA peptide 2 is boxed, and the peptides included in the alignment in panel *A* are indicated with an asterisk. Abbreviation: NHBA, Neisserial heparin binding antigen.

93.7–100% amino acid identities between strains (Figure 1; Table 1). Epitope mapping indicates that the human monoclonal antibodies 12E1 and 10C3 bind to the N-terminal region [48], while 5H2 interacts with a large, cross-reactive, conformational epitope in the C-terminal of NHBA-2 [49]. The regions bound by 12E1, 10C3, and 5H2 are conserved in the main gonococcal NHBA variants (Figure 1).

Bexsero Antigens Elicit Antibodies in Rabbits

To investigate the ability of antibodies raised to serogroup B meningococcal vaccine antigens to recognize gonococcal proteins, Western blot and ELISA analyses were performed using serum from rabbits immunized with either the OMV present in MeNZB and Bexsero (anti-OMV) or a combination of the recombinant antigens of Bexsero (anti-rMenB). Several bands in whole-cell lysates of *N. gonorrhoeae* strains WHO K, FA1090, and 1291 are recognized by the anti-OMV sera, and these proteins are consistent between the 3 gonococcal strains and are similar, but not identical, to proteins recognized in *N. meningitidis* (Figure 2A and 2B; Supplementary Figure S1). The anti-OMV sera had an ELISA titer of 128 000 to OMVs from *N. gonorrhoeae* 1291 (Supplementary Figure S1).

The anti-rMenB sera recognized all Bexsero protein antigens in *N. meningitidis* strain MC58 (NadA, fHbp, NHBA, GNA2091, and GNA1030), while only NHBA, GNA2091, and GNA1030 were recognized in *N. gonorrhoeae* (Figure 2A and 2C). NHBA was not recognized in trypsin-treated *N. gonorrhoeae*, confirming that NHBA is surface-exposed (Figure 2A and 2C). The detection of GNA2091 and GNA1030 were unchanged by trypsin treatment of *N. gonorrhoeae* (Figure 2A and 2C), indicating that they are located inside the cell, as previously described for *N. meningitidis* [34, 35]. The anti-rMenB sera recognized rNHBA from *N. meningitidis* MC58 and *N. gonorrhoeae* 1291 equally (Figure 2D), with ELISA titers of 2 048 000 to both proteins (Supplementary Figure S1).

Antibody Generation From Bexsero Vaccination in Humans

To investigate the ability of human, Bexsero-induced antibodies to recognize gonococcal proteins, Western blot and ELISA analyses were performed using serum from humans vaccinated with either 3 (0, 1, and 3 months) or 2 (0 and 1 months) doses of

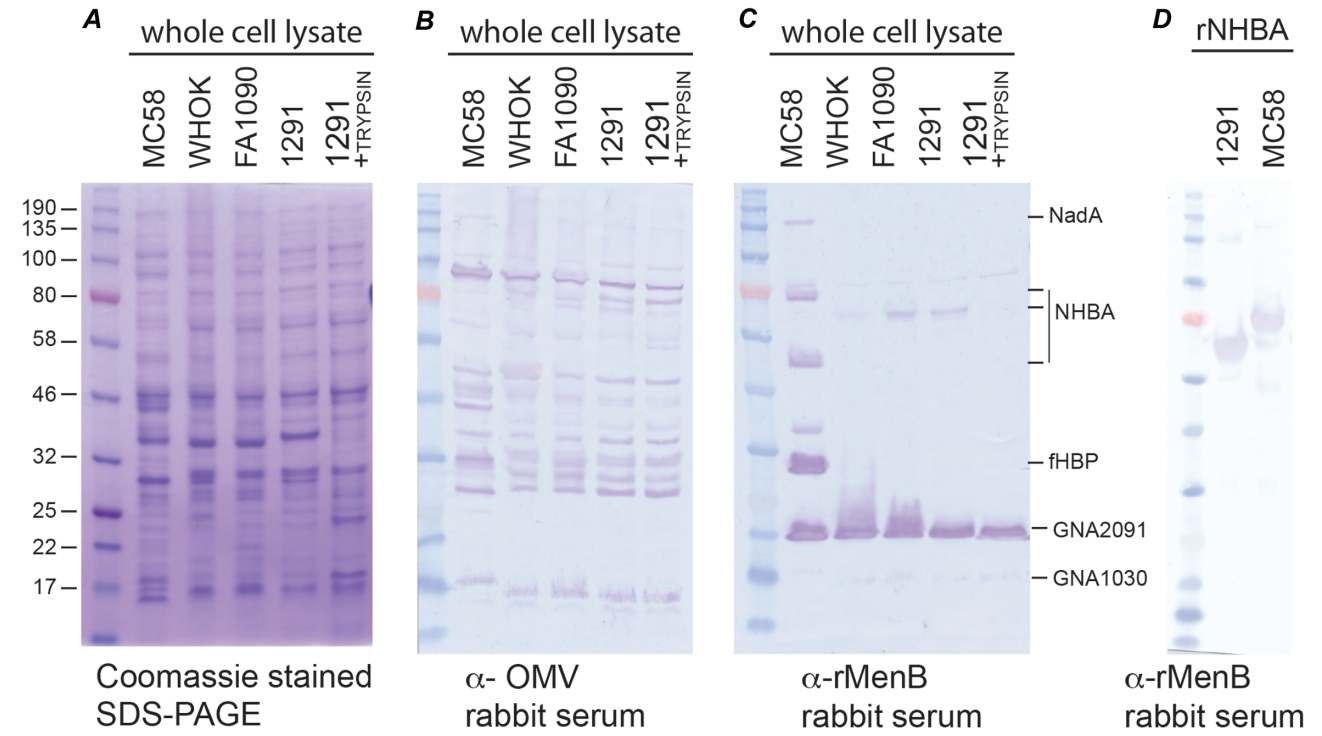

Figure 2. Reactivity of rabbit serum raised against Bexsero antigens to *Neisseria gonorrhoeae* antigens. *A*, Coomassie stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE); *B*, western blot with rabbit serum immunized with the NZ98/254 outer membrane vesicle component of Bexsero (α-OMV); and *C*, western blot with rabbit serum immunized to the recombinant protein component of Bexsero (α-rMenB). Samples shown are whole-cell lysates (equivalent to a final optical density at 600 nm of 5) from *Neisseria meningitidis* (strain MC58) and *N. gonorrhoeae* (strains WHO K, FA1090, 1291), and *N. gonorrhoeae* strain 1291, treated with trypsin for 60 min to remove surface proteins (1291+TRYPSIN). The protein ladder is shown on the left of each panel, with the protein sizes (kDa) on the far left. On the right of panel *C*, the recombinant proteins are indicated. For MC58 NHBA, the upper band is the full length NHBA protein, and the lower band is the fragment generated by NalP cleavage. For GNA1030, the protein is weakly expressed, and a digitally overexposed blot is shown in Supplementary Figure S1A, where GNA1030 is more evident. *D*, Western blot with α-rMenB rabbit serum against recombinant NHBA from *N. meningitidis* strain MC58 and *N. gonorrhoeae* strain 1291. Abbreviations: fHBP, factor H binding protein; NadA, Neisseria adhesin A; NalP, Neisseria autotransporter lipoprotein; NHBA, Neisserial heparin binding antigen; OMV, outer membrane vesicles; rNHBA, recombinant NHBA; SDS-PAGE, sodium dodecyl sulfate poly-acrylamide gel electrophoresis.

Bexsero. For the 10 individuals given 3 doses of Bexsero, there was no significant increase in the geometric mean ELISA titer (GMT) of the samples from pre-vaccination to 1 month post–dose 3 for gonococcal OMVs, which is likely due to the high pre-vaccine titers of some individuals. However, titers were significantly increased from pre- to post-vaccination for whole-cell *N. gonorrhoeae* (1.8-fold increased GMT, compared to 5.7-fold increase against whole-cell *N. meningitidis*) and gonococcal NHBA (34-fold increase; Table 2; Figure 3; Supplementary Figures S2–S5). A Western blot analysis of whole-cell lysates supports the ELISA data and shows reactivity to several gonococcal and meningococcal antigens with vaccinated, but not pre-immune, serum (Figure 3). There is a minimal amount of LPS present in detergent-extracted OMVs, which can induce a weak increase in antibodies to meningococcal LPS [45]. We see a minor increase in antibodies to meningococcal LPS, but no response to gonococcal LPS in sera from Bexsero-vaccinated individuals (Supplementary Figure S6).

An analysis of serum from individuals who received 2 doses of Bexsero was also performed, as this is the current recommended adolescent schedule in Australia, the United Kingdom, Canada, and the United States. Antibodies recognizing gonococcal OMV proteins were induced above the pre-vaccination baseline to similar levels at 1 month post–dose 1 and 1 month post–dose 2 (ELISA titer of 8000; Figure 4A). A Western blot analysis indicated that the pre-vaccination serum did not cross-react with gonococcal OMV proteins, while post–dose 2 sera reacted with several proteins (Figure 4B). Antibodies recognizing the gonococcal NHBA were induced after dose 1 (titer of 64 000) and to a very high-level at 1 month post–dose 2 (titer of 512 000; Figure 4C). **A** Western blot analysis indicated that pre-vaccination serum did not cross-react with gonococcal or meningococcal rNHBA (Figure 4C), while post–dose 2 sera reacted equally well with these rNHBA proteins (Figure 4D).

DISCUSSION

This study provides both bioinformatic and serological data on the potential of meningococcal vaccine antigens to generate an immune response that recognizes gonococcal proteins. These data provide experimental evidence for the concept that cross-reactive antibodies may be the mechanism that underlies the recent observation that the meningococcal serogroup B OMV vaccine MeNZB was associated with reduced rates of gonorrhoea [18]. The broad-spectrum serogroup B vaccine Bexsero, which contains the MeNZB OMV antigen plus 3 recombinant antigens (NadA, fHbp-GNA2091, and NHBA-GNA1030), is now licensed worldwide. In this study, we determined that there is a high level of amino acid identity between most of the major MeNZB/Bexsero OMV proteins and *N. gonorrhoeae* homologues, and that OMV-induced antibodies recognize gonococcal proteins. Furthermore, we have shown that NHBA is the only Bexsero recombinant protein antigen with a homologue in *N. gonorrhoeae* that is exposed on the surface of the bacteria and, therefore, accessible to vaccine-induced antibodies. We have also identified a high level of homology and cross-reactivity between the meningococcal and gonococcal NHBA proteins, which suggests that Bexsero may result in additional cross-protection against gonorrhoea, above that predicted for MeNZB.

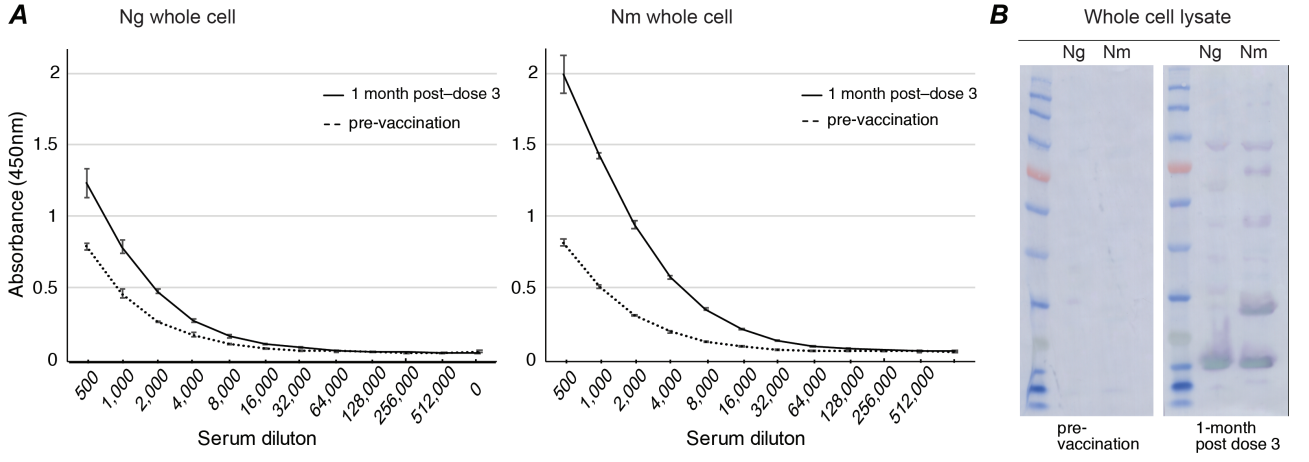

Figure 3. Reactivity of Bexsero-vaccinated human serum to whole-cell *Neisseria gonorrhoeae* (Ng) and *Neisseria meningitidis* (Nm). Reactivity of pooled Bexsero-vaccinated human serum from 10 donors vaccinated with 3 doses of Bexsero, at 0, 3, and 6 months. *A*, Enzyme-linked immunosorbent assay titration curves pre-vaccination (dashed line) and 1 month post–dose 3 (black line) against intact, whole-cell Ng 1291 and Nm MC58 are shown as the average absorbance (+/– standard deviation) at 450 nm versus reciprocal serum dilutions. *B*, Western blot analysis of whole-cell lysates shows recognition of several gonococcal and meningococcal antigens from post-vaccination, but not pre-vaccination, serum. Proteins recognized include those running at a molecular weight consistent with recombinant Bexsero antigens (Neisserial heparin binding antigen [NHBA], GNA2091, and GNA1030 in Ng and Neisseria adhesin A, NHBA, factor H binding protein, GNA2091, and GNA1030 in Nm). Abbreviations: Ng, *Neisseria gonorrhoeae*; Nm, *Neisseria meningitidis*.

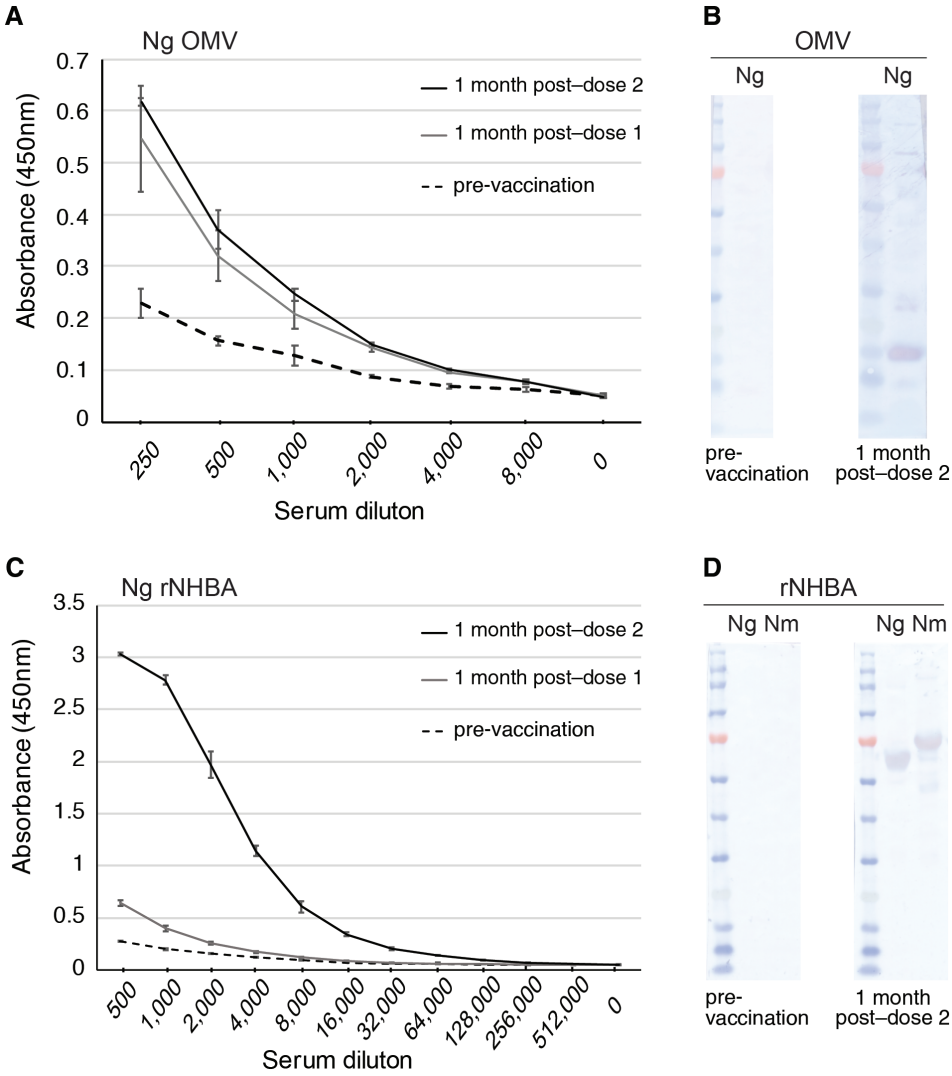

Figure 4. Reactivity of Bexsero-vaccinated human serum to *Neisseria gonorrhoeae* (Ng) outer membrane vesicles (OMVs) antigens and Neisserial heparin binding antigen (NHBA). Reactivity of Bexsero-vaccinated human serum from 1 donor vaccinated with 2 doses of Bexsero at 0 and 2 months to (*A* and *B*) Ng strain 1291 OMVs and (*C* and *D*) recombitant NHBA (rNHBA) from Ng strain 1291 or *N. meningitidis* (Nm) strain MC58. (*A* and *C*) ELISA titration curves of pre-vaccination (month 0, dashed line), 1 month post–dose 1 (month 1, grey line), and 1 month post–dose 2 (month 3, black line) are shown as the average absorbance (+/- standard deviation) at 450 nm versus reciprocal serum dilutions. Western blot analysis shows recognition of (*B*) several gonococcal OMV proteins and (*D*) rNHBA in post-vaccination, but not pre-vaccination serum. Abbreviations: Ng, *Neisseria gonorrhoeae* strain 1291; Nm, *Neisseria meningitidis* strain MC58; OMV, outer membrane vesicles; rNHBA, recombitant Neisserial heparin binding antigen.

The highly-variable PorA protein is the main antigen in meningococcal OMV vaccines that induces bactericidal antibodies, and it has long been considered likely that OMV vaccines do not protect against *N. meningitidis* strains expressing heterologous PorA types [23]. However, there is increasing evidence that some level of protection is provided against heterologous meningococcal strains, potentially due to minor OMV antigens, synergy between antigens, and/or a general immunomodulatory effect of OMVs induced by bacterial components such as LPS [24, 45, 50–52]. It is important to note that, although serum bactericidal activity is the established correlate of immune protection for *N. meningitidis*, the mechanisms of immune protection against *N. gonorrhoeae* are unknown and may involve cell-mediated killing or bactericidal, opsonophagocytic, and/or functional blocking activity of antibodies. Since *N. gonorrhoeae* rarely causes the invasive, life-threatening sepsis that is typical of meningococcal infection, then the sterilizing immunity conferred by meningococcal vaccines may not be required or appropriate to prevent gonococcal transmission and disease. Rather, a vaccine that is able to reduce mucosal colonization and transmission (ie, if antibodies that target a gonococcal adhesin are able to block bacterial adherence) or reduce pathology (ie, if antibodies that target a gonococcal virulence factor are able to reduce bacterial ascension to the upper genital tract) may be sufficient to reduce prevalence and disease burden [53]. Other studies have shown various levels of cross-reactivity or functional activity to *N. gonorrhoeae* in antibodies raised to meningococcal vaccines.

For example, mouse sera raised to an intranasal serogroup B Proteoliposome vaccine recognize *N. gonorrhoeae* by ELISA [54] and mouse sera raised to the meningococcal NHBA or NHBA-GNA1030 fusion protein can cross-react with *N. gonorrhoeae* F62 and induce complement deposition, as detected by flow cytometry [55]. Mouse sera raised to Bexsero or OMVs were able to reduce gonococcal adherence to epithelial cells. Mouse sera raised to either Bexsero, the Bexsero OMV or recombinant protein component, or NHBA, GNA1030, or GNA2091 alone showed serum bactericidal activity against *N. gonorrhoeae* FA1090. However, similar serum bactericidal activity (SBA) titers were seen for all these antigens despite whether they are surface localized on *N. gonorrhoeae* or not [55]. Mice immunized with Bexsero have been reported to have a significant reduction in the percentage of mice colonized and the bacterial burden through 7 days post-infection [56]. Mouse sera raised to native meningococcal OMVs have also been shown to induce SBA activity against *N. gonorrhoeae* FA1090, but no SBA activity was seen with serum from humans vaccinated with Bexsero in this study [57].

Irrespective of the functional immune response required for protection against gonorrhoea, there was a high level of immune reactivity of human, Bexsero-induced antibodies to *N. gonorrhoeae*. There was a significant increase in ELISA GMTs between pre– and post–Bexsero vaccine sera against gonococcal whole cells and, although the GMT to OMVs was not significantly increased, >50% of the samples did have an increased response to OMVs post-vaccination. Several individuals had high pre-immune titers against whole cells and OMVs, which was consistent with findings from the original study, where high baseline immunity against serogroup B meningococcal strains were seen (71% of individuals had pre-vaccination bactericidal antibody titers above the cut-off for PorA) [41], potentially due to prior meningococcal carriage or exposure in this at-risk laboratory cohort. The use of sera from laboratory workers is a potential limitation, and a larger study in the general population is needed. However, it is important to note that carriage rates of up to 35% are seen in young adults [58]. Of the recombinant antigens present in Bexsero that are not components of MeNZB, NHBA is potentially the only protein that may be able to provide an additive, protective effect towards *N. gonorrhoeae*. The Bexsero NHBA-2 shares 69% identity to the NHBA-527 variant from *N. gonorrhoeae* strain FA1090 and, as previously reported, the main difference between NHBA-2 and gonococcal NHBA peptides is due to a 189 nucleotide deletion in the N-terminal half of the gonococcal *nhba* gene [36]. Despite the presence of different NHBA variants, Western blot and ELISA data presented here show strong immune reactivity of anti-NHBA antibodies to *N. gonorrhoeae*, with a 34-fold increase in the ELISA GMT between pre– and post–Bexsero vaccine sera against the gonococcal NHBA antigen. This cross-reactivity between NHBA variants is supported by a recent analysis of meningococcal strains circulating in the United States, which demonstrated the immune reactivity of anti-NHBA antibodies with 99.5% of isolates, irrespective of the NHBA genotype [59]. This meningococcal antigen typing system (MATS) analysis predicted that NHBA provided high (84–100%) coverage of strains that expressed 1 of the 8 major NHBA variants present in the US isolates [59], including NHBA-10, 20, 21, and 29 (shown in Figure 1), which share a similar level of identity to the NHBA-2 and to the 3 major gonococcal NHBA variants (72–79% identity for meningococcal vs 67% identity for gonococcal variants). NHBA induces antibodies that have serum bactericidal activity against a diverse collection of meningococcal strains [32, 33], are opsonophagocytic [60, 61], and are able to block the adherence of *N. meningitidis* to epithelial cells [62]. NHBA antibodies may have similar functional activities against *N. gonorrhoeae*.

Mathematical modelling of hypothetical gonococcal vaccines indicated that a vaccine efficacy of 31%, as predicted for MeNZB [18], could decrease gonorrhoea prevalence by >30% in the 20 years after vaccine implementation, if vaccine-induced protection could be maintained for longer than 10 years [53]. A higher level of vaccine efficacy would be optimal and may potentially be afforded by Bexsero, due to the additional NHBA component. Given that antibiotic-resistant gonococcal infections are a rapidly-emerging health problem worldwide, vaccine development is an increasing priority [9] and, if untreatable gonorrhoea [63] becomes widespread, then a modestly-effective vaccine would be better than no vaccine. Ideally, a gonococcal-specific vaccine consisting of a combination of promising candidate gonococcal antigens (as recently reviewed [1, 10]) should enter human clinical trials as soon as possible to determine whether a higher vaccine efficacy can be achieved. However, until now there have been limited tools to enable the discovery of what is required to induce a protective immune response, and there has been little to no clinical progress towards a gonococcal vaccine in the last 3 decades [1, 10]. The landmark finding that individuals vaccinated with MeNZB were significantly less likely to contract gonorrhoea compared with unvaccinated controls [18] represents the first time that any vaccine has been associated with protection against gonorrhoea in humans. This observation, and the data on cross-reactivity outlined here, provides a new opportunity to progress gonococcal vaccine development, guided by the human immune response to the vaccine-mediated presentation of antigens that are common between *N. gonorrhoeae* and the closely-related *N. meningitidis*. Further work is needed to identify the full set of gonococcal targets recognized by Bexsero-induced antibodies and the mechanism(s) of protection against gonorrhoea that are mediated by these antibodies or by other components of the immune system. However, this study provides both a new framework to advance gonococcal vaccine development and firm evidence to justify new human trials to investigate the potential level of Bexsero-induced protection against gonorrhoea.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted

materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. R. B. performs contract research on behalf of Public Health England for GlaxoSmithKline, Pfizer, and Sanofi Pasteur. K. L. S. worked for Novartis Vaccines on the development of Bexsero, from 2006 to 2012. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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