

[0107] These results demonstrate the ability of the combined vaccine to induce bactericidal (protective) antibodies against a broad range of group B strains and potentially strains of other serogroups as well.

[0108] Analysis of the bactericidal antibodies using a bactericidal depletion test demonstrated that antibodies to all three sets of antigens were involved in killing at least some of the test strains. In some cases, it appeared that antibodies to more than one antigen were involved and acted together to produce bactericidal activity against a given strain.

[0109] Additional groups of mice were vaccinated with NOMV vaccine prepared from isogenic mutants of strain 8570 HOPS-G1. The mutant strains differed in their expression of PorA. Two mutants expressed a single PorA (one or the other of the two in the multivalent vaccine strain) and the third was a PorA knockout mutant expressing no PorA protein. Bactericidal titers induced by each of the four strains against several different test strains are shown in Table 10.

TABLE 10

Test Strain	Mutant of 8570 HOPS-G1 from Which NOMV Vaccine Was Prepared			
	8570 (P1.19, 15 and P1.22, 14)	8570 (P1.19, 15)	8570 (P1.22, 14)	8570 APorA
8570	256	256	256	256
44/76	256	256	256	256
B16B6	1	1	1	1
3576	2	8	8	8
9547	2	4	4	2
2981	64	1	16	1
6557	32	1	128	1

[0110] For the first five test strains in Table 10, the PorA expression had no effect on the titer of bactericidal antibodies induced by the vaccine. For the last two strain, which both express P1.14, the presence of the P1.14 epitope in the vaccine correlated with the capacity of the respective serum to kill the strain. This demonstrates that antibodies to PorA are involved in the observed killing for some strains. For other strains such as the homologous strain and strain 44/76 other antigens are responsible for most of the bactericidal activity. This was demonstrated by analysis with the bactericidal depletion assay. Results of one such assay are given in FIG. 16. The results shown in FIG. 17 demonstrate that antibodies to LOS and FHBP (GNA1870) were involved in the killing of strain 8570 by antiserum to PorA knockout mutant of 8570 HOPS-G1.

1. A vaccine comprising native outer membrane vesicles obtained from at least two meningococcal strains that have been genetically modified to provide broad based protection, wherein the native outer membrane vesicles include three different sets of antigens based on PorA, LOS, and conserved outer membrane proteins; wherein the genetically modified strains have been modified to provide enhanced safety based on inactivation of lpxL1, synX, and lgtA genes; and wherein at least one of the genetically modified strains expresses at least two different PorA subtype proteins or subtype epitopes.

2. The vaccine of claim 1 where the LOS expressed by each strain has a different LOS core structure and has an alpha chain consisting of glucose and galactose.

3. The vaccine of claim 1 where each strain expresses at least two different PorA subtype proteins or subtype epitopes

which are chosen based on the most prevalent of PorA subtypes among group B case isolates.

4. The vaccine of claim 1 where a different conserved surface protein with demonstrated capacity to induce bactericidal antibodies is over-expressed in each strain and are taken from the group consisting of FHBP variant 1, FHBP variant 2, FHBP variant 3; NadA; App; NspA; TbpA and TbpB.

5. A combination of NOMVs from three genetically modified, antigenically diverse *Neisseria meningitidis* strains, wherein at least one of the strains is selected from:

(1) H44/76 HOPS-DL which has the following genetic modifications or characteristics:

inactivation of the genes synX, lpxL1, and lgtA;

insertion of a second porA gene (subtype P1.7-1,1) in the place of opaD;

increased expression of NadA; and

stabilized high expression of Opc and PorA;

(2) 8570 HOPS-G₄L which has the following genetic modifications or characteristics:

inactivation of the genes synX, lpxL1, and lgtA;

insertion of a second porA gene in place of opaD;

increased expression of factor H binding protein variant 1; and

stabilized high expression of PorA and Opc; and

(3) B16B6 HPS-G₂A which has the following genetic modifications or characteristics:

inactivation of the genes synX, lpxL 1, and lgtA;

insertion of a second porA gene in place of opaD;

increased expression of factor H binding protein variant 2; and

stabilized high expression of PorA and Opc.

6. The combination of vaccine strains of claim 5 wherein strain H44/76 HOPS-DL was derived from the ET-5 wild type strain H44/76 (B:15: P1.7,16: L,3,7:P5.5,C).

7. The combination of vaccine strains of claim 5 wherein strain 8570 HOPS-G₇L was derived from the ET-5 wild type strain 8570 (B:4: P1.19,15: L3,7v: P5.5,11,C).

8. The combination of vaccine strains of claim 5 wherein strain B16B6 HPS-G₂L is derived from the ET-37 wild type strain B16B6 (B:2a:P1.5,2: L2:P5.1,2,5).

9. The vaccine of claim 1 where the NOMV are prepared without exposure to detergent or denaturing solvents from packed cells or from spent culture medium.

10. The vaccine of claim 1 where the vaccine is suspended in 5% glucose as an excipient.

11. The vaccine of claim 1 where the NOMV are combined with one or more adjuvants selected from the group consisting of aluminum hydroxide, aluminum phosphate, MF 59, CPG-ODN, and MPLA.

12. A method of using the vaccine of claim 1 administered intramuscularly and/or intranasally for immunization against meningococcal disease.

13. A method of using the vaccine of claim 1 administered intramuscularly and/or intranasally for immunization against group B meningococcal disease.

14. A vaccine composition against meningococcal disease comprising native outer membrane vesicles (NOMVs) from one or more genetically modified strains of *Neisseria meningitidis*, wherein the one or more genetically modified strains has been modified by:

i. inactivation of the synX gene,

ii. inactivation of the lpxL1 gene,