

[0102] In addition, expression of *OpcA* was stabilized by curing the phase variation associated with its gene. This was done as described for strain 8570 HOPS-G1 by breaking up the poly-G string in its promoter in Example 1. The *IgtA* gene was interrupted as in Example 1 producing a truncated LOS. A phase variant of the resulting strain expressing the L8 immunotype was selected by colony blotting with an L8 specific monoclonal antibody. This genetically modified strain was designated 44/76 HOPS-D as shown in FIG. 18. The two additional strains were characterized to confirm stability of all the genetic modifications and stocks of each were frozen down.

Example 10

Preparation of NOMV Vaccine from Strains B16B6 HPS-G2 and 44/76 HOPS-D

[0103] The three genetically modified strains were used to prepare laboratory lots of NOMV vaccine compositions. The strains were grown in Catlins modified medium as one liter cultures in Fernbach flasks on a rotary shaker. The cells were harvested by centrifugation, weighed and the cell paste frozen. The cell paste was thawed and used to prepare NOMV following essentially the same procedure as described for the clinical lot of vaccine from strain 8570 HOPS-G1 as

described in Example 2. The process was scaled down and ultracentrifugation twice at 225,000×g for 60 min at 2-8° C. to remove nucleic acids and all soluble, non-vesicle material.

Example 11

Immunization of Mice with Complete Multivalent Vaccine

[0104] Groups of ten CD-1 mice were vaccinated intraperitoneally with two µg of NOMV vaccine from each genetically modified vaccine strain (6 µg total for the combined vaccine with NOMV from three strains). Three doses were given at 0, 4, and 8 weeks. Blood was drawn pre-vaccination and 2 weeks following the last vaccination (at 10 weeks).

[0105] Sera from individual mice were tested for bactericidal antibodies against the homologous strains, and pooled serum from each group of 10 mice was tested against a panel of 14 heterologous group B strains and 1 group C strain expressing a broad range of different subcapsular antigens.

[0106] The combined multivalent vaccine induced a geometric mean 1:256 titer against each of the three vaccine strains and a 4-fold or greater increase in bactericidal antibodies against 13 of the heterologous strains. Two of the test strains were not killed in spite of having an antigen shared with one of the vaccine strains. The bactericidal titers observed against the panel of strains are given in the Table 9.

TABLE 9

Bactericidal Titers of Pooled Mouse Sera against a Diverse Panel of Test Strains						
Bactericidal		Titer of Pooled Serum from Mice Vaccinated with Indicated Vaccine				
		B1 + B2 + B3	B2 + B3	B1	B2	B3
Test Strain	Antigens Expressed					
44/76	B:15:P1.7,16:P5.11,C:L3,7	256	256	256	256	1
8570	B:4:P1.19,15:P5.5:L3,7v	256	256	256	256	2
816136	B:2a:P1.5,2:P5.1a:L2	256	256	1	1	256
9162	B:15:P1.7-2,3:P5.10,11:L3,7*	16	16	8	2	1
M1080	B:1,19:P1.1:P5.3:L1,3,7	2	1	1	1	1
3576	B:NT:P1.22-1:L3,7	128	128	4	2	16
8047	B:2b:P1.5,2:L3,4,7	64	128	1	1	128
9547	B:4:P1.4:L1	256	256	128	2	64
531	B:2a:P1.5,2:P5.1a,1b,4,5:L3,7	256	256	256	1	256
7608	B:2b:P1.5,2:P5.2,c:L4	256	128	1	1	128
6940	B:8,19:P1.NT:L1	4	4	1	1	1
1901	B:8,19:P1.NT:P5.C:L1,3,7	256	32	128	1	8
99M	B:2a:P1.2:P5.1a,1b,5:L3,7	512	512	16	8	256
6275	B:2a:P1.2:P5.1a,4,5:L3,7	—	512	32	1	512
126E	C:8,19:P1.5,2:L1:P5.c	256	256	4	2	64
2981	B:8,19:P1.14:L1	32	8	4	64	2
M4720	B:19:P1.22,14:L2	1	1	1	1	1
6557	B:17:P1.14:L1(3,7)	32	16	1	32	16

Vaccine Code:

B1 = 44/76 HOPS-D NOMV

B2 = 8570 HOS-G1 NOMV

B3 = B16B6 HPS-G2 NOMV