Exploring the expression levels of recombinant antigens and the murine response to Novartis recombinant MenB investigational vaccine

Rachel Bigwood, Ian Feavers, Hannah Chan and Caroline Vipond

Department of Bacteriology, NIBSC, Blanche Lane, Potters Bar, EN6 3QG, UK

INTRODUCTION

To combat the lack of a successful vaccine against group B meningococcal disease, a new vaccine formulation from Novartis V & D, based on three recombinant antigens, two of which are fused to additional recombinant proteins, is being developed. NIBSC is in the process of developing a batch release testing regime for the vaccine, and produced serum from a small immunogenicity study. The recombinant proteins are outer membrane proteins consistently found on the surface of the meningococcus. The proteins are presented in the vaccine as two fusion proteins and a single antigen. Fusion protein 1 (FP-1) consists of Neisseria heparin binding antigen (NHBA) conjugated to GNA1030, fusion protein 2 (FP-2) of factor H binding protein (fHbp) conjugated to GNA2091 and the single antigen NadA. Outer membrane vesicles (OMVs) made from strain NZ98/254 were added to the vaccine to broaden coverage regarding ST 41/44 strains and to provide additional outer membrane proteins to the vaccine (1).

The aims of this project were to assess the phenotypic expression of fHbp, NHBA and NadA across a panel of diverse strains. This panel of strains was then used to investigate the cross reactive antigenic response elicited in mice vaccinated with either the OMV or the investigational vaccine.

RESULTS

Table 1. Overview of whole cell ELISA results. ELISA plates were coated with $5x10^7$ cells per well. Pooled serum from NIH mice vaccinated with investigational vaccine, OMV (NZ98/254), FP-1, FP-2 or NadA and boosted at day 28, was serially diluted from 1 in 500 to 1 in 64000. The level of reaction against each strain was scored: ++++ strong reaction, +++ medium reaction, ++ weak reaction, + very weak reaction. An immune response was observed against the twelve strains using whole cell ELISAs to evaluate serum from animals vaccinated with the investigational vaccine.

A strong response was seen against five strains which reacted with serum raised against FP-2 and are variants of the serosubtype P1.7. Two of the strains also reacted with FP-1 and one strain with NadA.

A medium response was observed against three strains all of which reacted against serum raised against FP-2 and one reacted with serum raised against FP-1.

A weak reaction was observed against two strains, which also reacted with serum raised against FP-2 and one strain also reacted with serum raised against NadA.

Finally a very weak reaction was observed against two strains which did not show a response with serum raised against FP-1, FP-2 or NadA. (The sera had already been shown to contain antibodies specific for the recombinant proteins and the single antigen).

Membrane prep number	Strain Number	Serogroup	Clonal Complex	PorA	PorB	FetA	fHbp	Response of serum in mice raised against:				
								Investigational Vaccine	NZ98/254 OMVs	Fusion protein 1	Fusion protein 2	NadA
1	Z6413	C	ST-8 complex/Cluster A4	P1.18-1,3	ND	F4-1	ND	+	++	-	-	-
2	Z4673	B	ST-41/44 complex/Lineage 3	P1.7-2,4	NT	F1-5	1	++++	++++	-	+	-
3	Z6412	B	ST-8 complex/Cluster A4	P1.5-1,2-2	ND	F1-4	ND	+	+	-	-	-
4	Z1054	A	ST-5 complex/subgroup III	P1.20,9	4,21	F3-1	1	+++	+++	-	+	-
5	Z1275	A	ST-1 complex/subgroup I/II	P1.5-1,10-1	4,21	F1,7	1	+++	++++	+	+++	-
6	MC58	B	ST-32 complex/ET-5	P1.7,16-2	15	F1-5	1	+++	++	-	++	-
7	FAM18	C	ST-11 complex/ET-37 complex	P1.5,2	2a	ND	ND	++	+++	-	+	+
8	M06 240022	B	ST-269 complex	P1.18, 25-1	ND	ND	1	++	+++	-	++	-
9	NZ98/254	B	ST-41/44 complex/Lineage 3	P1.7,4	4	ND	1	++++	++++	-	+	-
10	H44/76	B	ST-32 complex/ET-5	P1.7,16	15	F3-3	1	++++	++++	+	++++	-
11	LNP20404	B	ST-32 complex/ET-5	P1.7,16	14	ND	1	++++	++++	+	+++	+
12	LNP24584	B	ST-32 complex/ET-5	P1.7,16	14	ND	1	++++	++++	-	+++	-

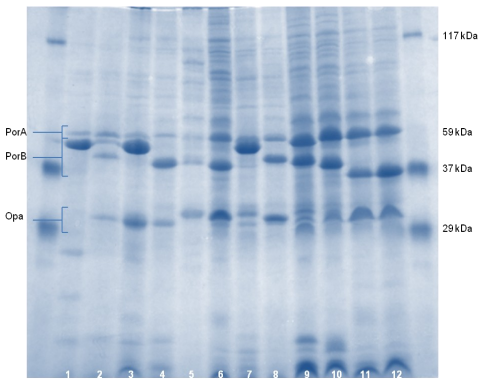

Figure 1. Outer membrane protein preparations from the strain separated on a 12% 1D SDS-PAGE gel.

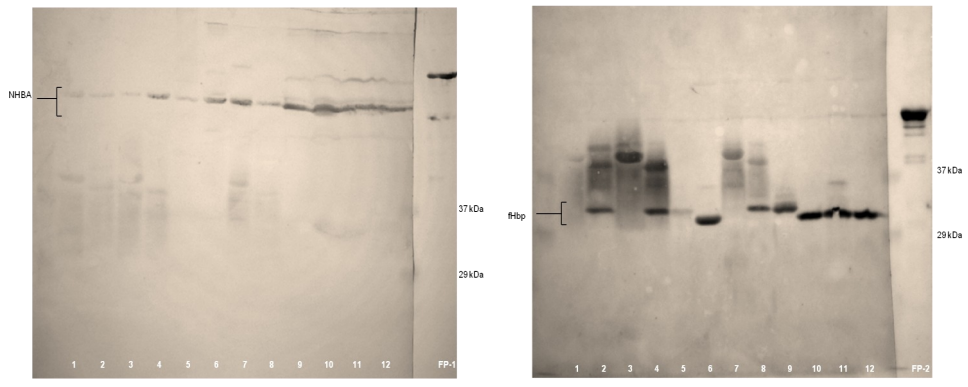

Figure 2. Immunoblot using rabbit serum raised against FP-1 and FP-2 against the strain panel. The proteins from a 1D SDS-PAGE gel were transferred to a nitrocellulose membrane and expression levels of the native proteins in the strains were examined. A response was observed with serum raised against FP-1 directed at a protein approximately 60kDa, likely to be NHBA. A strong response was observed when blotting with the serum raised against FP-2. The band on the blot at approximately 31 kDa is presumed to be fHbp. A strong reaction can be seen in numerous strains at approximately 40 kDa. No response was detected using serum directed against NadA (image not included).

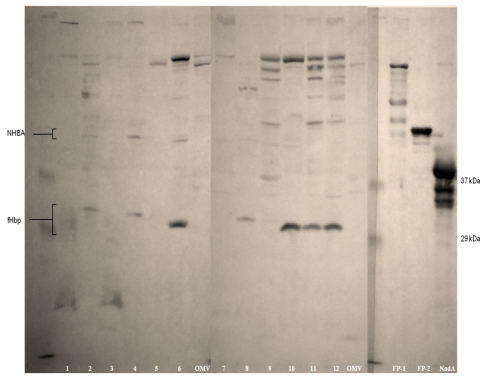

Figure 3. Immunoblot of the panel of strains probed with serum from mice given the investigational vaccine. In strains MC58, M06 240022, H44/76, LNP20404, LNP24584 there is a response at approximately 31 kDa. In strains Z4673, Z1054, MC58, NZ98/254 and LNP20404 there are responses at approximately 50 kDa. Finally in a number of strains, including Z1275, MC58, M06 240022, NZ98/254, H44/76, LNP20404 and LNP24584 there are responses against a number of high molecular weight proteins.

CONCLUSIONS

The level of response of the mouse serum raised against the investigational vaccine varied across a diverse strain panel. These differences are a reflection of the variability in the surface structures and differing levels of phenotypic expression of antigens across the strain panel. The weaker response for NadA may result from the degradation of the native protein conformational epitopes. However, the whole cell ELISA results suggest that the investigational vaccine is able to elicit a strong response against isolates expressing fHbp variant 1 and PorA serosubtypes P1.7, and a reaction was seen against these isolates on the immunoblot using investigational vaccine sera.

The recombinant protein components of the vaccine, FP-1, FP-2 and NadA were evaluated. FP-2 immunogenicity serum elicited the strongest and most broadly cross reactive response against the strains. In comparison serum raised against FP-1 and NadA evoked a weak response against the strain panel. Immunoblots of the recombinant protein sera show bands at 40 kDa that is likely to be a cross-reaction with PorB. A strong reaction occurs at this position in the strains that lack a fHbp band, suggesting that the presence of fHbp reduces the cross-reaction with PorB.

When the vaccine was formulated with the OMV a response directed against fHbp was observed in seven of the 12 strains. In six of strains a possible response against NHBA was seen in comparison with a reaction against three strains in whole cell ELISAs with serum directed against FP-1 alone. This suggests that the OMV enhances responses against FP-1.

The observation of a response directed at a number of high molecular weight proteins suggest that the immunogenicity of this vaccine is based on multiple antigens and not solely on the three recombinant antigens. The importance of the responses to the different antigens depend on the phenotype of the strain.

Future work

- Further investigation of the enhancing properties of OMVs
- Quantitative immunoblotting to determine expression levels of protein.

REFERENCE

1. **Giuliani, M. *et al*.** (2006). A universal vaccine for serogroup B meningococcus. Proc. Natl. Acad. Sci. U. S. A **103**:10834-10839.