Development and assessment of pre-clinical immune responses to a heat shock protein-enriched meningococcal disease vaccine

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INTRODUCTION

Heat shock proteins (Hsps) are molecular chaperones that bind polypeptides, prevent aggregation and support protein folding. Expression of Hsps is increased by stress (e.g. heat, anoxia, nutrient starvation) and may comprise up to 15% of total cell protein. Hsps can also induce innate immune responses, activate dendritic cells and deliver peptides leading to antigen presentation and development of adaptive immunity. We have developed a meningococcal disease vaccine prepared from heat-shocked cultures of *Neisseria meningitidis* or *N. lactamica* and assessed immune responses against a panel of diverse meningococcal strains.

METHODS

Preparation of vaccines

Hsp-enriched vaccines were prepared from *N. meningitidis* (MC58) and *N. lactamica* (Y92-1009). Bacteria were grown in shake flask cultures in Frantz medium and heat shocked at 44^{o}C for 2h. Bacteria were harvested by centrifugation, broken by homogenisation and filtered supernatant diluted and applied to a Capto Q chromatography column. Fractions enriched in Hsp65 and Hsp70 were eluted and pooled to produce the vaccines. For further details see Bailey et al., Poster, IPNC 2010.

Meningococcal mouse challenge model

Groups of ten mice were immunised with 10µg protein on days 0, 21 and 28, and challenged with *N. meningitidis* (44/76-SL) on day 35.

Opsonophagocytosis assay (OPA)

The granulocytic cell line HL60 was used. Azide-killed meningococci labelled with the fluorescent dye DilC were used as the target bacteria; IgG-depleted human plasma was used as the complement source and a single-point determination of OP activity was made at a serum dilution of 1:20; all assays were performed in duplicate. Flow cytometry was used to determine the percentage of HL60 cells taking up the labelled meningococci and the intensity of fluorescence uptake; the data were expressed as the signal of test antibody minus the signal from the no antibody, complement-only control (FI-C').

Antibody-mediated C5b-9 deposition assay

Azide-killed meningococci were incubated with test antibody and IgG-depleted human plasma for 30mins at room temperature, washed twice, and mouse-anti-human C5b-9 (AlexaFluor 647) used to measure deposition by incubation for 20min at 4^{0}C. Flow cytometry was used to detect the percentage of meningococci showing fluorescence (complement binding) and the intensity of that fluorescence (level of complement). The data was expressed as the signal of test antibody minus the signal from the no-antibody, complement-only control (FI-C').

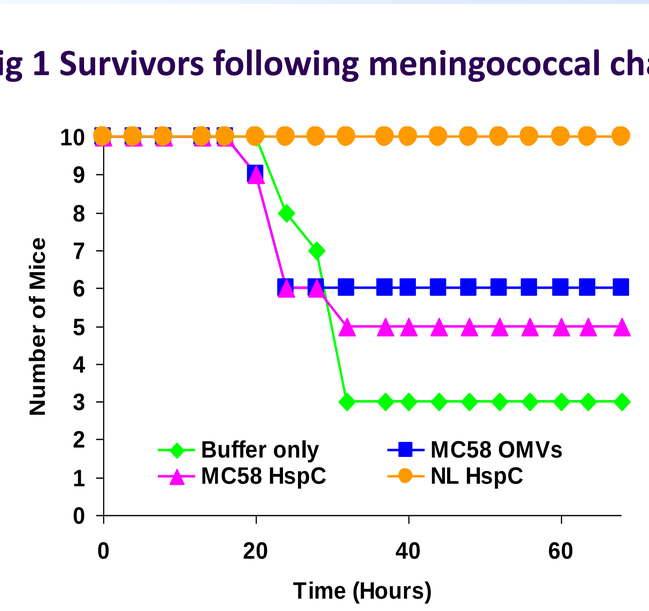
Serum bactericidal activity(SBA)

Human serum was used as a source of complement with titres expressed as the reciprocal of the dilution giving ≥50% SBA killing at 60min, compared with the control.

RESULTS

Protection against meningococcal challenge provided by *N. meningitidis* and *N. lactamica* heat shock protein-enriched vaccines was assessed in a mouse model of meningococcal septicaemia (Fig 1). The meningococcal OMV vaccine and Hsp-enriched vaccine provided similar protection but complete protection against this challenge dose was provided by the *N. lactamica* Hsp-enriched vaccine. Opsonophagocytosis (Fig 2)and antibody-mediated C5b-9 deposition (Fig3) were assessed against a panel of strains (Table 1) and for the majority of strains the *N. lactamica* Hsp-enriched vaccine provided the greatest responses. SBA activity up to titres of 1:16 were detected against strains M01-240101, M01-240355 and 44/76. No SBA was detected against M01-240013 and M01-240149.

Fig 1 Survivors following meningococcal challenge



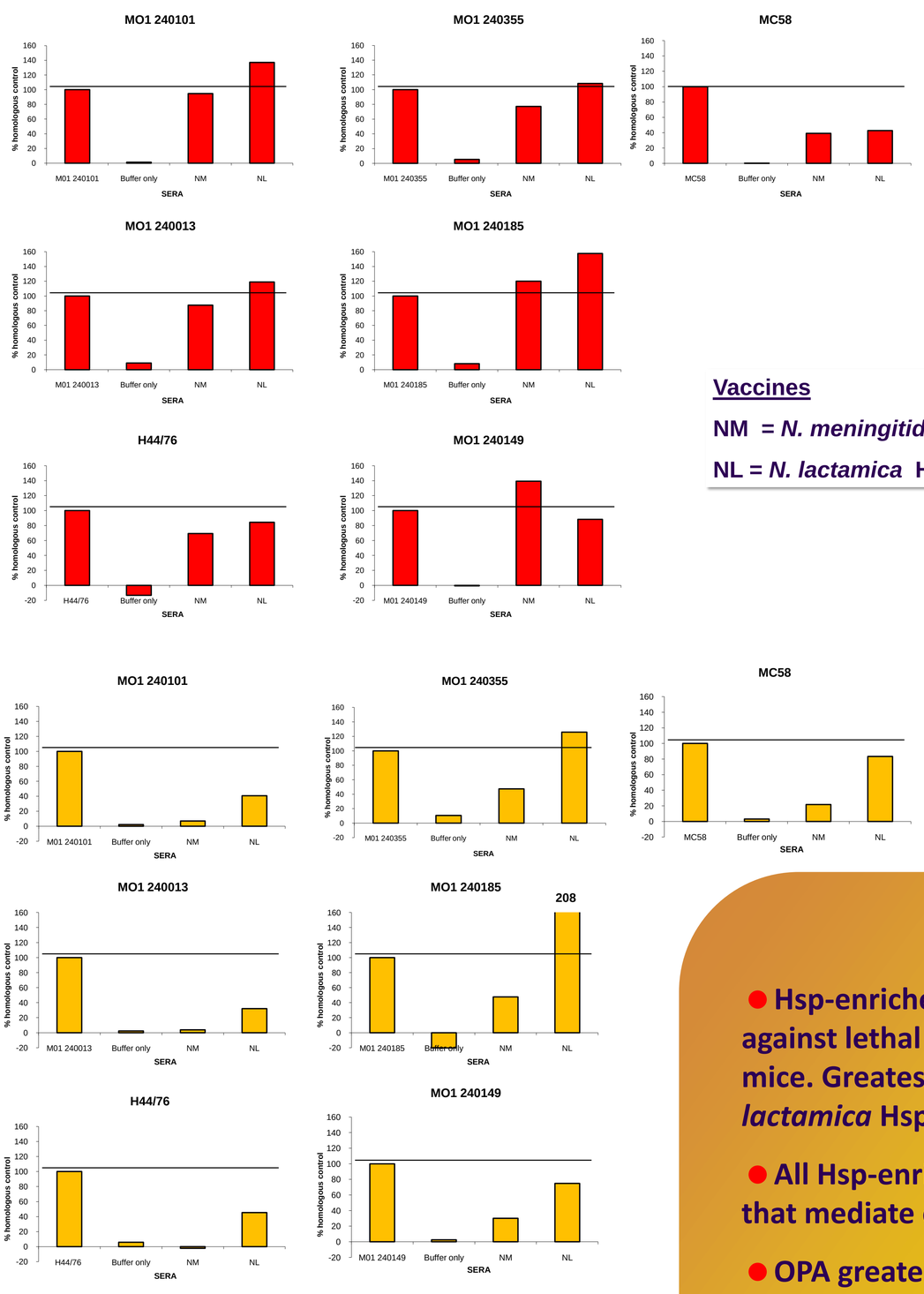


Fig 2 Opsosonophagocytosis expressed as a % of the homologous strain OMV serum response

Vaccines

NM = *N. meningitidis* Hsp-enriched vaccine

NL = *N. lactamica* Hsp-enriched vaccine

Fig 3 Antibody-mediated C5b-9 deposition expressed as a % of the homologous strain OMV serum response

Table 1. Meningococcal strains

Strain	Serogroup	Serotype	Serosubtype	Sequence Type	Clonal Cluster
MC58	B	15	7,16	74	32
44/76-SL	B	15	7,16	32	32
M01-240101	B	NT	19-1,15-11	1049	269
M01-240013	B	NT	22,9	275	269
M01-240149	B	4	7-2,4	41	41/44
M01-240185	B	2a	5-1,10-8	11	11
M01-240355	B	1	22,14	213	213

Summary

- **Hsp-enriched vaccines provide protection against lethal meningococcal challenge in mice. Greatest proection was seen with an *N. lactamica* Hsp-enriched vaccine**
- **All Hsp-enriched vaccines elicit antibodies that mediate opsonophagocytosis**
- **OPA greater than that determined for the homologous strain OMV sera was observed for 5 out of 7 strains**
- **Hsp-enriched vaccines elicited antibodies that mediate C5b-9 membrane attack complex deposition.**
- **Greater OPA and C5b-9 deposition was seen for most strains with the *N. lactamica* – based vaccine**
- **SBA observed with 3/5 meningococcal strains with the *N. lactamica* vaccine**