Neisseria lactamica induces anti-*Neisseria meningitidis* B cell responses

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Introduction & Aims

Introduction

Colonisation with *Neisseria lactamica* (Nlac) prevents *Neisseria meningitidis* (Nmen) colonisation and disease. If the mechanism underlying this effect was elucidated it could be exploited to develop novel strategies to protect against Nmen colonisation and disease. We theorised that an adaptive cross-reactive immune mechanism, independent of SBA, may be implicated in this protection and performed a Nlac controlled human infection experiment to test this hypothesis.

Aims

1. To establish if pharyngeal colonisation with Nlac induces Nlac-specific B cell responses that are cross-reactive with Nmen.
2. To assess whether the magnitude of Nlac-specific B cell responses induced following Nlac colonisation were associated with Nlac colonisation density.

Methods

- 31 participants were randomised to receive intra-nasal inoculation with 10^5 colony-forming units (CFU) of Nlac (Y92-1009) suspended in 1ml phosphate buffered saline (PBS) (intervention), or 1ml PBS (control).
- Nlac and Nmen colonisation status was assessed at baseline and at 7-, 14- and 28-days post-inoculation by culture of oropharyngeal swabs and nasal wash. Nlac colonisation density was measured in nasal wash.
- Nlac (Y92-1009)-specific and Nmen (H44/76)-specific IgA-secreting and IgG-secreting plasma cell (B_{PLAS}) and IgG memory B cell (B_{MEM}) frequencies were quantified in blood at baseline and post-inoculation time points using enzyme-linked immunospot assays (ELISpot).
- Nlac-specific and Nmen-specific IgG titers were measured in plasma using enzyme-linked immunosorbent assays (ELISA).

Results

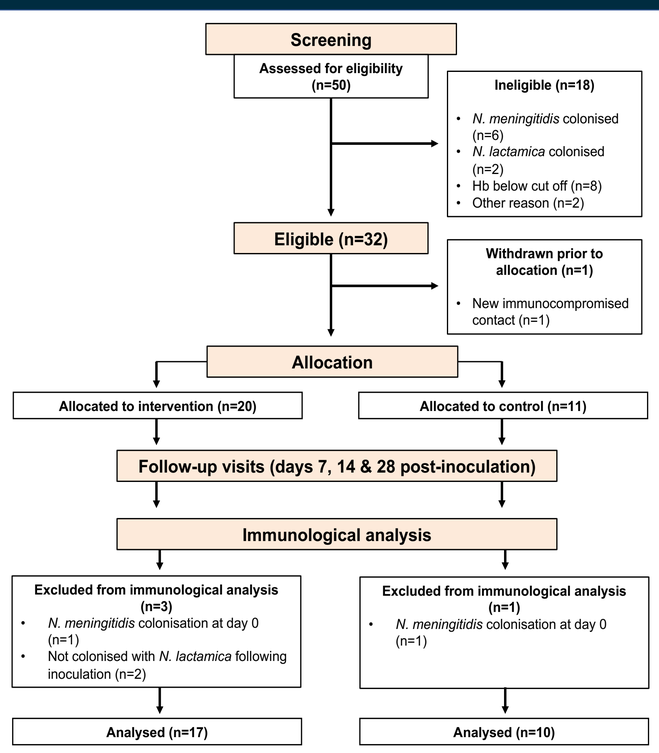

Figure 1. Study flow diagram showing allocation to groups, study completion and participants included in the immunological analyses. Hb – haemoglobin concentration in whole blood.

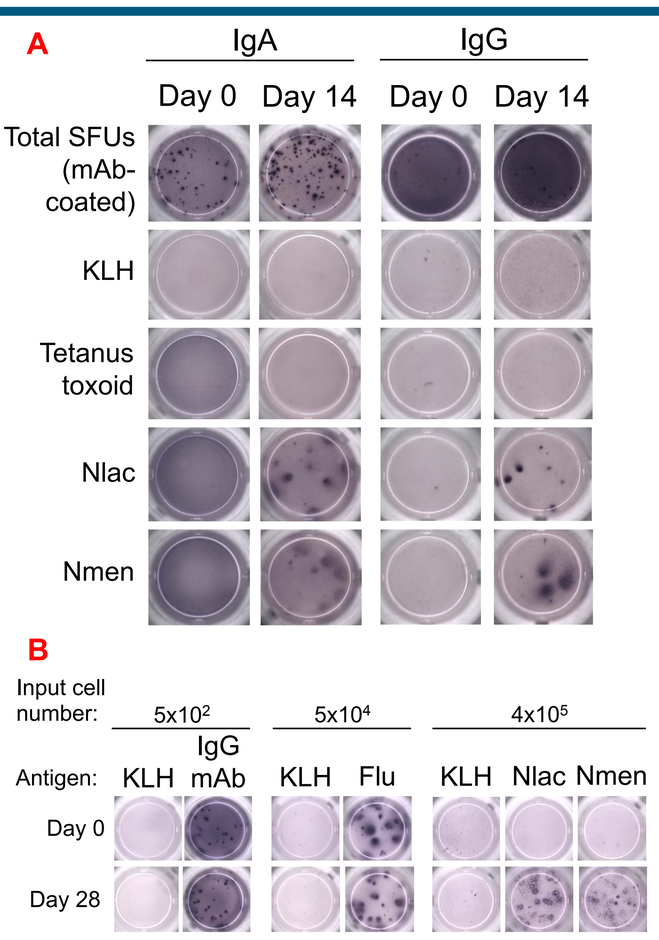

Figure 2. Representative examples of IgA and IgG B_{PLAS} and IgG B_{MEM} ELISpot assays for Nlac-inoculated and colonised participants. For the IgA- and IgG-secreting B_{PLAS} ELISpot assays (**A**), 2 x 10^5 peripheral blood mononuclear cells (PBMCs) were seeded in duplicate wells coated with anti-human IgG or IgA monoclonal antibodies (mAb) (total SFUs), keyhole limpet haemocyanin (KLH) (negative control), tetanus toxoid (bystander control), and deoxycholate-extracted outer-membrane vesicles (dOMV) derived from both Nlac Y92-1009 (Nlac) and Nmen H44/76 (Nmen). For the IgG B_{MEM} ELISpot assay (**B**), PBMCs were polyclonally stimulated for 5 days with CPG DNA, IL-2 and IL-10 prior to seeding into triplicate wells coated with KLH, anti-human IgG mAb, influenza haemaglutinin (Flu), Nlac-dOMV and Nmen-dOMV. Following an 18-hour incubation, alkaline phosphatase-conjugated anti-IgA or anti-IgG secondary polyclonal antibodies were added prior to development with BCIP substrate. One spot-forming unit (SFU) was considered representative of one B_{PLAS}/B_{MEM} for enumeration purposes.

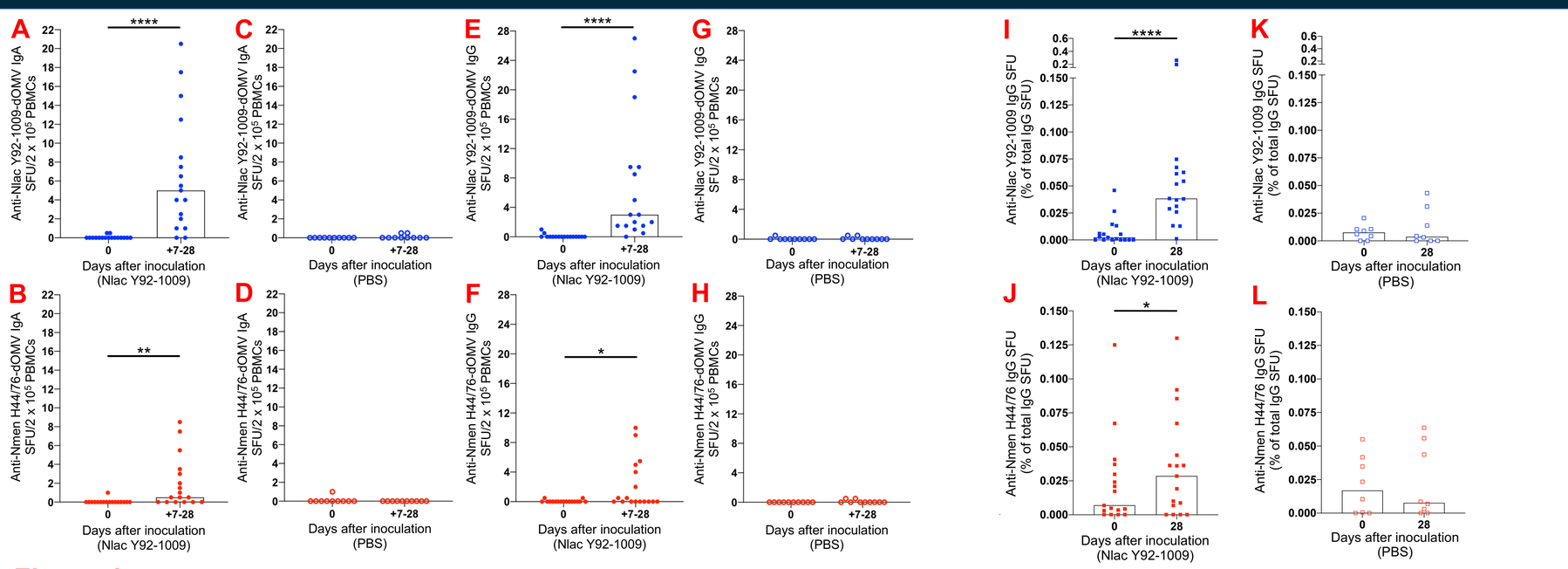

Figure 3. Colonisation with Nlac induces anti-Nlac and anti-Nmen B_{PLAS} and B_{MEM} responses. PBMCs were derived from Nlac-colonised and PBS-inoculated participants and assessed by ELISpot for the presence of IgA-secreting (**A-D**) and IgG-secreting (**E-H**) B_{PLAS}, and IgG B_{MEM} (**I-L**), specific for Nlac Y92-1009-dOMV (**A, C, E, G, I, K**) and Nmen H44/76-dOMV (**B, D, F, H, J, L**). B_{PLAS} and B_{MEM} were visualised as SFU, having adjusted for non-antigen-specific SFU by subtraction of SFU enumerated in KLH-coated wells. For B_{PLAS} data, the highest number of SFU per 2 x 10^5 PBMCs is shown (day +7-28) for each antigen *vs.* baseline. Bars indicate median. *$P \leq 0.05$, **$P \leq 0.01$, **** $P \leq 0.0001$ by Wilcoxon matched-pairs signed rank test (n = 17 Nlac-colonised participants, n = 10 PBS-inoculated participants).

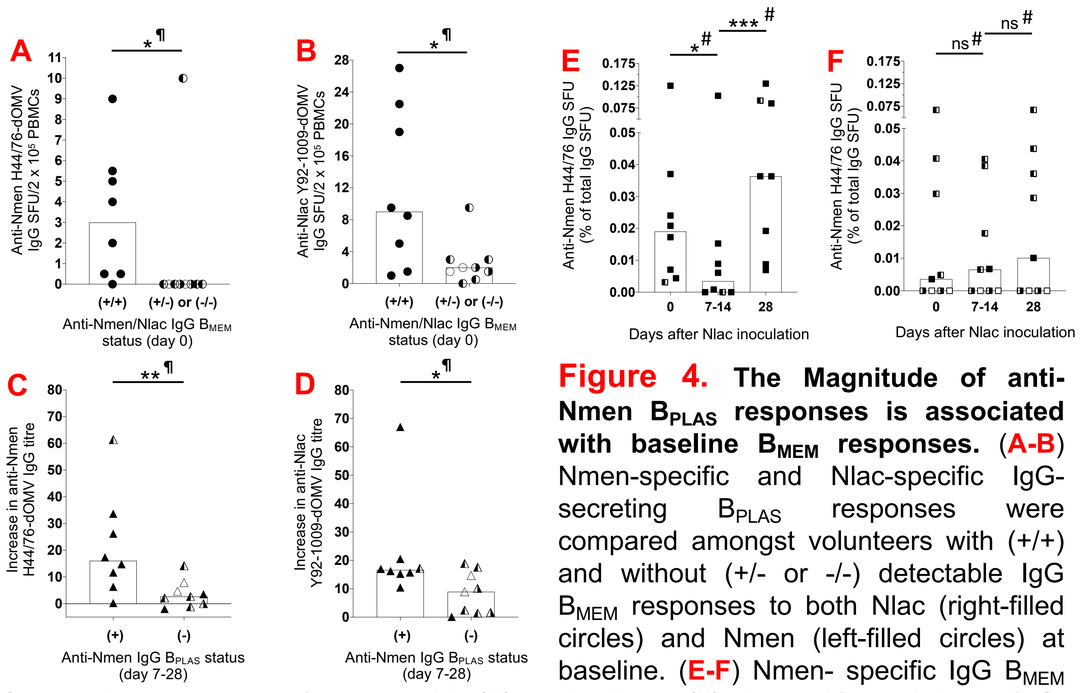

Figure 4. The Magnitude of anti-Nmen B_{PLAS} responses is associated with baseline B_{MEM} responses. (**A-B**) Nmen-specific and Nlac-specific IgG-secreting B_{PLAS} responses were compared amongst volunteers with (+/+) and without (+/- or -/-) detectable IgG B_{MEM} responses to both Nlac (right-filled circles) and Nmen (left-filled circles) at baseline. (**E-F**) Nmen- specific IgG B_{MEM} frequencies amongst volunteers with (**E**) and without (**F**) detectable anti-Nmen IgG-secreting B_{PLAS} responses, comparing day 0 and day 28 frequencies with the lowest frequency measured on either day 7 or day 14. (**C-D**) Increase in anti-Nmen and anti-Nlac IgG titre between days 0 and 28 amongst participants with (+) and without (-) a detectable anti-Nmen IgG-secreting B_{PLAS} response. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ by Mann-Whitney test (¶) or Kruskal-Wallis test with Dunn's multiple comparisons test (#).

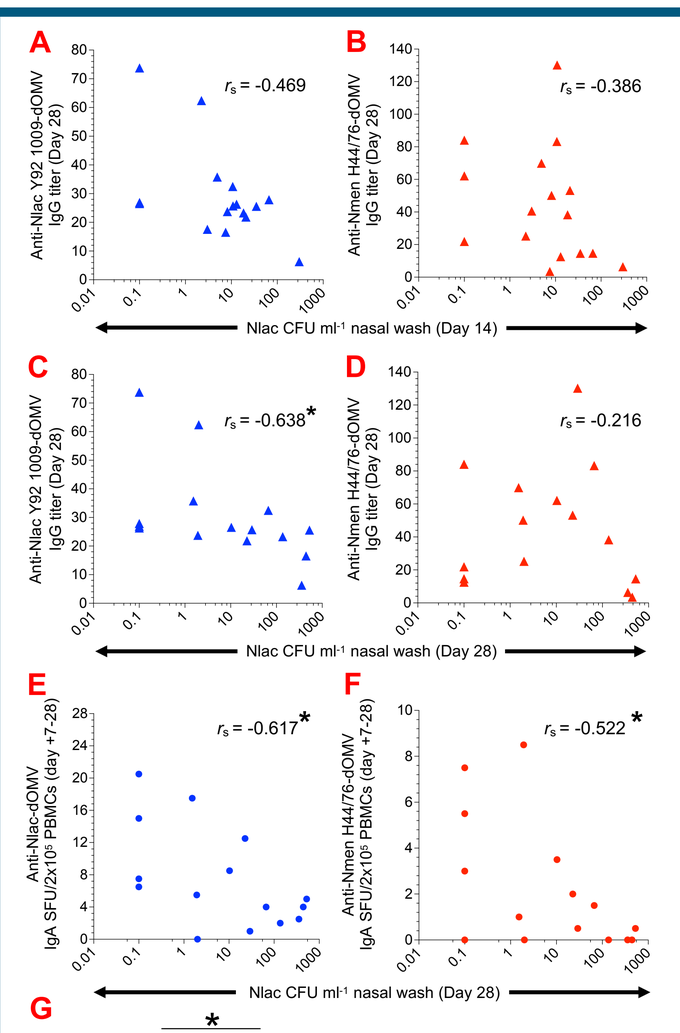

Figure 5. Nlac-specific B_{PLAS} responses and IgG titers are associated with Nlac colonisation density. Day-28 IgG titers (**A-D**) and peak IgA-secreting B_{PLAS} responses (**E-F**) were plotted against Nlac colonisation density (Nlac CFU ml^{-1}, nasal wash) on days 14 and 28 post-inoculation for each Nlac-colonised participant and correlations assessed using Spearman's Rho (r_s) (*$P \leq 0.05$). (**G**) Area under the curve (AUC) Nlac colonisation density calculated using Nlac CFU ml^{-1} data derived from nasal wash at days 7, 14 and 28 post-inoculation amongst participants with (+/+) and without (+/- or -/-) detectable IgG B_{MEM} responses specific to both Nlac (right-filled circles) and Nmen (left-filled circles) at baseline. *$P \leq 0.05$ by Mann-Whitney test.

Conclusions & Future Work

- Colonisation with Nlac induces IgA-secreting and IgG-secreting B_{PLAS} and IgG B_{MEM} with specificity to Nlac and Nmen (**Figure 3**).
- That Nmen-specific IgG B_{PLAS} frequencies were higher amongst Nlac colonised participants where Nlac-specific and Nmen- specific IgG B_{MEM} were both detectable at baseline (**Figure 4A**) suggests that Nlac colonisation may have boosted pre-existing cross-reactive B_{MEM} responses. This theory is further supported by the observation that Nmen-specific IgG B_{MEM} frequencies reduced amongst Nlac-colonised participants where anti-Nmen IgG-secreting B_{PLAS} responses were induced (**Figure 4E**).
- The observation that anti-Nlac IgG titers and anti-Nlac IgA-secreting B_{PLAS} frequencies negatively correlated with Nlac colonisation density (**Figures 5C & 5E**) suggests that the magnitude of these responses may play a role in controlling Nlac colonisation density.
- If the generation of anti-Nmen B_{PLAS} or antibody induced by Nlac colonisation is responsible for the protective effect afforded by Nlac on Nmen then we would predict protection would only be afforded in those where anti-Nmen responses were induced. We intend to test this hypothesis using the Nlac controlled human infection model.

Acknowledgements

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