



Comparison of complement sources in the meningococcal serogroup B serum bactericidal antibody assay

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Introduction

- Serogroup B strains of *Neisseria meningitidis* are an important cause of meningitis, both an endemic and epidemic disease that is a major health problem in various parts of the world (Rosenstein *et al.*, 2001).
- The surrogate of protection against *Neisseria meningitidis* serogroup B (MenB) is the serum bactericidal antibody (SBA) assay (Borrow *et al.*, 2006).
- Pioneering studies utilised a human complement source (Goldschneider *et al.*, 1969), but problems in procuring human complement of sufficient quality and quantity have resulted in baby rabbit complement being first recommended by the WHO (WHO, 1976) and subsequently used in serogroup A, C, Y and W135 assays (Masklanka *et al.*, 1997).
- However, it is infeasible to use baby rabbit complement in the MenB SBA assay due to elevated titres caused by low avidity anti-MenB capsular IgM antibody in test sera (Zollinger *et al.*, 1983).
- A previous study demonstrated that colominic acid could be used as an adsorbent to remove anti-capsular antibody and improve the correlation in SBA titres between baby rabbit and human complement. However, results varied and were not identical to those achieved with human complement so is not recommended (Findlow *et al.*, 2007).
- Recently other factors have come to light which may account for elevated titres given by baby rabbit complement. Including the addition of human factor H to the MenC SBA assay (using baby rabbit complement) resulted in a reduction in SBA titre compared with baby rabbit complement alone (Welsch *et al.*, 2008).
- For interlaboratory standardisation of the MenB SBA assay, a commercially available non-human complement source remains an attractive alternative.
- Other species such as larger animal species may give results which closer resemble those achieved with human complement
- We therefore investigated the use of porcine and bovine complement in the MenB SBA assay.

Aim

The aim of this investigation was to determine if bovine and porcine complement could be used as an alternative to human complement in the MenB SBA assay.

Methodology

SBA methodology

- Pre- and post-vaccination sera (n=150) from an adult study of the New Zealand OMV vaccine MeNZB™ were assayed in the SBA assay against NZ 98/254 (B:4:P1.7-2,4) with human complement (h), three lots of bovine complement (b1, b2, b3), porcine complement (p) and rabbit complement (r).
- SBA titres were expressed as the reciprocal of the final serum dilution giving $\geq 50\%$ killing at 60 minutes (T60)

Statistical analysis

- Distribution of SBA titres and average differences in SBA titre step gained using non-human and human complement were calculated.
- Correlations between SBA titre gained using non-human and human complement were determined (Spearman's rank correlation test).
- Geometric mean titres (GMTs) with 95% confidence intervals (CI) were calculated (Wilcoxon matched pairs test).
- Proportions of subjects with ≥ 4 fold rises in SBA titre from pre- to post-vaccination were calculated (Fisher's exact test).

Figure 1 Distribution of SBA titres produced with human and non-human complement against target strain NZ 98/254

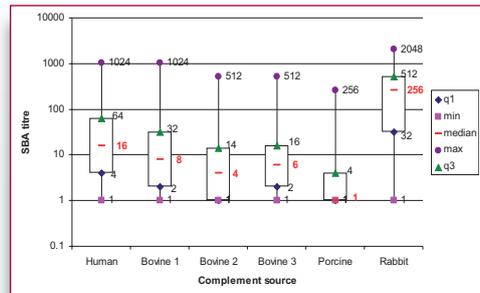


Figure 2 Average differences in SBA titre steps gained using non-human complement compared to human complement against target strain NZ 98/254

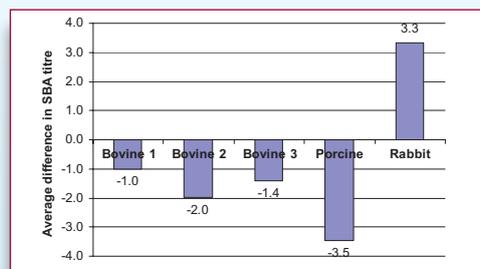


Figure 3 Correlation of SBA titre (95% confidence intervals) between human complement and non-human complement against target strain NZ 98/254

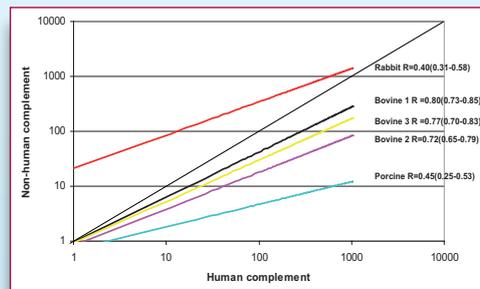


Figure 4 SBA GMTs (95% confidence intervals) pre- and post-three doses of MeNZB as determined with different complement sources against target strain NZ 98/254

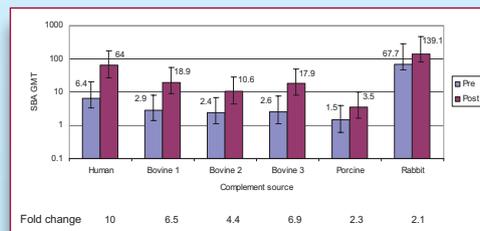
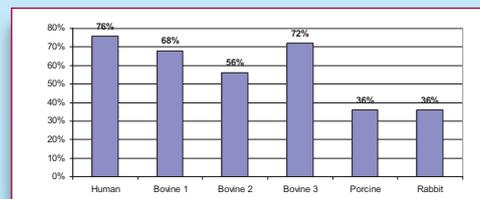


Figure 5 Proportions of subjects showing ≥ 4 fold rises in SBA titre between pre and post-three doses of MeNZB with different complement sources against target strain NZ 98/254



Summary of results

- In general, bovine and porcine complement gave lower SBA titres while rabbit complement gave higher SBA titres compared to human complement with the same sera against target strain NZ 98/254 (Figure 1).
- The average difference in reduction of SBA titre step compared to human complement were 1.01, 1.97, 1.40 and 3.46 for b1, b2, b3 and porcine, respectively; while rabbit complement gave a 3.34 fold rise in SBA titre step (Figure 2).
- Correlation coefficients of 0.80, 0.72, 0.77, 0.45 and 0.40 were achieved between human complement and b1, b2, b3, porcine and rabbit complement, respectively (Figure 3).
- Significant increases in SBA GMT from pre- to post-three doses were achieved with all complement sources ($p < 0.001$) with the exception of rabbit complement for strain NZ 98/254 ($p = 0.188$). A 10 fold change in SBA titre from pre- to post-three doses was achieved against NZ 98/254 with human complement, whereas fold changes ranged from 2.1 to 6.9 with non-human complement sources (Figure 4).
- A ≥ 4 fold rise in SBA titre from pre- to post-vaccination is regarded as seroconversion. Only b1 and b3 achieved this significant sensitivity reflecting the true outcome compared to human complement against strain NZ 98/254 (Figure 5).

Conclusions

- We demonstrated stronger correlations between bovine and human complement compared to porcine and rabbit complement.
- Bovine complement could be a potential source for MenB SBA assay.

Future work

- Further investigations are needed to examine the consistency of this correlation between bovine complement and human complement in SBA assay against other strains. Particularly as differences were demonstrated between the three sources we investigated.
- To evaluate and determine a putative protective SBA titre for bovine complement.
- To investigate the effect of addition of human factor H to MenB SBA assay on SBA titre with non-human complement.

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