Developing a standardised opsonophagocytosis killing assay (OPkA) for Group B Streptococcus

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INTRODUCTION

Group B Streptococcus (GBS) is a major cause of neonatal sepsis and meningitis worldwide and can also cause sepsis in pregnant women. Thus a maternal vaccine could be effective in preventing GBS infant disease by placental IgG transfer as well as protecting the mother against GBS sepsis. It has been shown that high titres of naturally occurring serotype specific maternal antibody to capsular polysaccharides (CPS) are associated with a reduced risk of GBS disease in neonates. However, there is no consensus on the concentration of anti-GBS antibody required for protection. This is due to a lack of standardised ELISAs to measure IgG concentration and no standard OPkA to assess antibody function. As studies are designed to determine immune correlates of protection against GBS disease by assessment of natural antibody in case/control studies, it is important that both antibody quantity and function is assessed. Understanding this relationship will be required for immune assessment of clinical trials of new GBS vaccines. As part of a consortium funded by the Bill and Melinda Gates Foundation, we are developing a standard multiplex ELISA and a standard OPkA that can be used to evaluate large panels of sera from mothers and infants.

OPkA development

<table>
<thead>
<tr>
<th>Assay parameters investigated</th>
<th>Phagocytosis stage</th>
<th>Viable count</th>
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<tbody>
<tr>
<td>● Incubation time: 7.5–60 mins, 7.5 mins gave lower titres, 60 mins gave fluctuations in survival curves</td>
<td>● Complement added at opsonisation or phagocytosis stage killing observed in Ab-independent controls of certain strains</td>
<td>● OPkA buffer: gelatin &amp; FCS conc. investigated no difference</td>
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<tr>
<td>● Incubation temp: 25°C vs 37°C no difference</td>
<td>● Baby rabbit complement at 3, 6 or 12.5% controls counts all acceptable between 80-250 at 12.5%</td>
<td>● Incubation time: 7.5 – 60 min no difference</td>
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<td>● Celt:bacteria ratio of 100:1 or 400:1 no difference</td>
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Final method

20µl 1x10⁴ CFU per well GBS + 20µl serum doubling dilution from 1:32
Incubate 30min at 37°C total volume of 100µl with OPkA buffer (HBSS + Mg²⁺ + Ca²⁺ + 0.1% gelatin)
OPkA buffer gelatin & FCS conc.
30min at 37°C
Plate10µl per well on COH agar & incubate overnight & count colonies
Titres determined as 50% killing compared to no serum control at assay end

OPkA with SHRS against all serotypes

- Sixteen human sera from healthy adults were tested on three separate occasions; one operator on two separate days and a second operator on one day.
- Titres varied from <1:32 to >16384.
- The reciprocal titres for the three occasions were log-transformed and the means, SD and coefficient of variation (CV) values determined.
- For all sera the CV values were below 13%
- Mean CV was 6.0%.

Reproducibility of OPkA with serum panel (n=16)

- Multiple GBS strains of five serotypes; Ia, Ib, II, III and V were obtained from PHE Respiratory and Vaccine Preventable Reference Unit, Colindale. Prof. Carol Baker, Baylor College of Medicine, Houston or PHE NCTC.
- IgG-binding of homologous and heterologous CPS-conjugate vaccine sera was determined by flow cytometry. These pooled human vaccine sera for each serotype are the current GBS Standard Human Reference Sera (SHRS) and were obtained from Prof. Carol Baker. Strains were eliminated if binding to the homologous SHRS was low.
- Strains were also assessed for their performance in the OPkA and a small number were eliminated as they showed killing with baby rabbit complement and differentiated HL60 cells in the absence of antibody.

SHRS contains cross reactive IgG

- IgG-binding with anti-Ia, Ib, III and V SHRS to the five GBS serotypes was determined by flow cytometry.
- IgG binding to four serotype III strains is shown.
- All SHRS contain IgG that binds to GBS of other serotypes.
- This is likely due to both anti-CPS and anti-protein antibodies developed following asymptomatic carriage of GBS.

SUMMARY

- We have made good progress in developing an OPkA for assessment of anti-GBS immunity that can be used in studies to determine immune correlates of protection.
- We have selected invasive clinical isolates for each of the five serotypes based on their expression of CPS and their performance in the OPkA.
- OPkA titres obtained with SHRS and volunteer sera are reproducible.
- We will continue to optimise the OPkA to ensure we have a robust and reproducible assay that can be transferred and used in multiple settings to allow comparison of results between studies.