Understanding the pyrogenic response to Bexsero (4CMenB) vaccine

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INTRODUCTION
The multi-component 4CMenB vaccine, Bexsero, is for use against Neisseria meningitidis serogroup B. Bexsero is a part of the infant vaccination schedule in the United Kingdom. As the vaccine often elicits a fever response it is recommended that paracetamol is administered alongside vaccination.

Bexsero has four antigenic components; three recombinant proteins, and Outer Membrane Vesicles (OMV) from N. meningitidis. OMV contain several reactogenic components including outer membrane proteins (OMP) and lipopolysaccharide (LPS).

Bexsero is known to contain LPS, a potent fever causing agent (pyrogen). In addition, other non-endotoxin pyrogens are present in the OMV contributing to the overall fever response.

This has been illustrated in the MonoCyte Activation Test (MAT), used to monitor the total pyrogen content of vaccine batches. Non-endotoxin pyrogens affect the overall pyrogenic response observed when the vaccine is tested, resulting in non-parallel dose-response curves compared to purified LPS (Figure 1).

While it is thought that non-endotoxin pyrogens play a role in the fever response to Bexsero, it is not yet known which components are responsible or how they modulate the pyrogenic response. In this investigation we aim to identify the pathways involved in the innate immune response to Bexsero, providing an understanding of how several pyrogens contribute to the overall fever response.

MATERIALS AND METHODS
HEK-293 cell lines engineered to express specific Toll-like Receptors (TLRs) and a NFκB reporter (InvivoGen) were used to identify pyrogenic agonists in the samples. TLR-NFKB signalling induced expression of Secreted Embryoidal Alkaline Phosphatase (SEAP), detected by colourimetric assay (figure 2).

Three TLR cell lines were selected for investigation; TLR4 (LPS sensing), TLR2 (lipoprotein, peptidoglycan sensing), and TLR9 (CpG rich DNA sensing).

TLR2 forms functional dimers with either TLR1 or TLR6, with specific agonists for each dimer. Neutalising monoclonal antibodies against TLR1 and TLR6 were used to selectively inhibit signalling. Vaccine samples, and positive controls for each dimer were tested at a constant EC50 dose, and a dilution series of anti-TLR MAb was applied generating an inhibition curve.

Total pyrogenicity values were determined by MAT as outlined in the European curve.

RESULTS
Vaccine samples tested showed a detectable response via TLR2 signalling. The dose-response curves generated were parallel to agonists of both TLR2-TLR1, and TLR2-TLR6 dimers. It was therefore important to investigate which of the two dimers was responsible for activation of the pyrogenic signalling response.

Use of neutralising antibodies specific to TLR1 and TLR6 showed there was signalling via the TLR2-TLR1 dimer after vaccine exposure. Anti-hTLR6 neutralising antibodies had no effect on TLR2 signalling in response to the vaccine (figure 4).

TLR2-TLR1 signalling levels detected in vaccine samples tested ranged between values equivalent to 165-550 ng/ml of Pam3CSK4 (TLR2-TLR1 positive control).

No signalling via TLR9 was observed in response to the vaccine. However, it was discovered that the vaccine masked detection in samples spiked with known quantities of CpG rich DNA (data not shown). This effect is likely to also occur in MAT testing and it is therefore expected that TLR9 signalling does not contribute to the overall pyrogenic response measured.

CONCLUSIONS
The use of TLR specific cell lines provides reproducible estimates for activity relative to characterised controls. The use of spiked vaccine samples have shown the results generated to be accurate and not influenced by other product components (data not shown), with the exception of TLR9. The strong correlation between TLR4 signalling and overall pyrogenicity confirms LPS as the primary factor responsible for the fever response to the vaccine. However, several relatively pyrogenic samples tested show low LPS content and a high TLR2 signal, which could be a compensatory factor resulting in a higher overall pyrogenic response.

It is yet unclear which particular components are responsible for the TLR2 response, though several known agonists are present in the OMV, such as PorA and native lipoproteins.

REFERENCES:
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