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## INTRODUCTION

Current vaccines targeting *N.meningitidis*, *S.pneumoniae* and *H.influenzae* use the polysaccharide capsule that surrounds the organisms as the vaccine antigen. However, the biochemical nature of the polysaccharides prevent them recruiting T-cell help in the generation of protective B-cell immunity. This results in poor immune responses in infancy and limited long-term protection. In order to overcome this, the polysaccharides are conjugated to T-cell dependent carrier proteins. These are thought to recruit T-cell help in the generation of polysaccharide specific B-cell responses. Whether the polysaccharide specific responses thus generated are the same as those generated against the carrier proteins is unknown and is important as it is likely to affect the long term protective efficacy of the vaccines. We have compared the response of Men C polysaccharide (CPS) specific B-cells to the response of B-cells specific to tetanus toxoid (TT), the carrier protein, following conjugate vaccination of healthy adults.

## METHODS

### 1. Subjects, vaccine and blood samples:

18 adult volunteers were vaccinated with a UK licensed CPS - TT conjugate vaccine (NeisVacC, Baxter). All had received a Men C conjugate vaccine more than 4 years previously and no intervening TT vaccine. Blood samples were taken prior to, and at time points up to six weeks following vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation.

### 2. Plasma cell ELISpot assay:

96 well plates were coated with CPS or TT. PBMCs were added in triplicate to the wells and cultured in standard medium at 37°C overnight. The cells were then washed off and spots, representing the site of CPS or TT specific plasma cell, were enumerated using a standard enzyme substrate reaction.

### 3. Memory B-cell ELISpot assay:

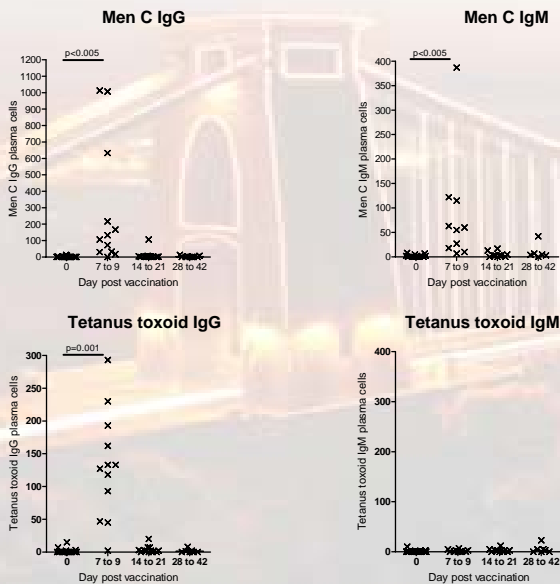
Fixed protein A bearing *S.aureus* cells (SAC) and IL-2 were initially confirmed, by flow cytometric based cell sorting, to stimulate the differentiation of isotype switched memory B-cells into plasma cells. PBMCs were subsequently cultured for 6 days with SAC and IL-2 and the number of plasma cells present at the end of culture, indicating the relative initial number of memory B-cells were enumerated with an ELISpot assay.

### 4. T-cell proliferation

The proliferation of cells in response to antigenic stimuli was assessed by measuring the incorporation of tritiated thymidine to 100ul aliquots of cells in triplicate over 18 hours.

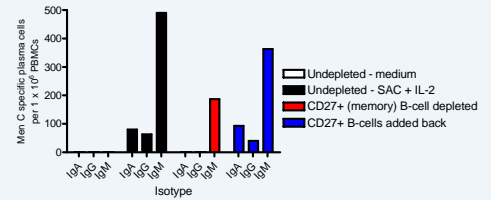
## RESULTS

### 1. Plasma cell responses in the circulation following vaccination are short lived. IgG alone is produced in response to TT whereas IgG and IgM are produced in response to CPS



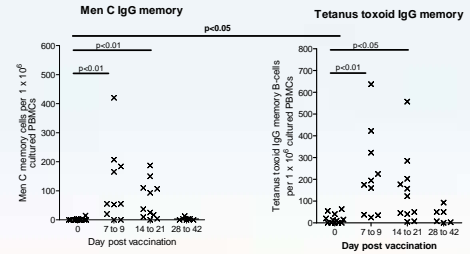
**Figure 1: IgM and IgG secreting plasma cells specific for CPS and TT following conjugate vaccination.** Despite previous vaccination no plasma cells are identified to either CPS or TT prior to booster vaccination. Following vaccination plasma cells are only identified briefly in the circulation and return to baseline levels by day 14. While IgG secreting plasma cells alone are generated in response to TT, both IgG and IgM secreting cells are generated against CPS.

### 2. IgG secreting memory B-cells differentiate into plasma cells following culture with SAC and IL-2



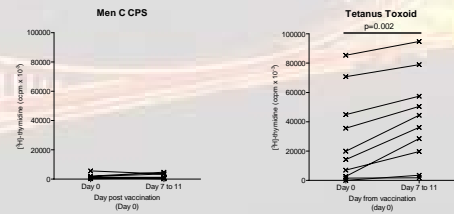
**Figure 2: The effects of depleting CD27+ memory B-cells on the generation of plasma cells through culture with SAC and IL-2.** Culturing PBMCs obtained following vaccination with SAC and IL-2 results in the generation of IgA, IgG and IgM secreting plasma cells specific for CPS. The IgA and IgG responses are lost when memory B-cells are removed although the IgM responses are preserved. This confirms that SAC + IL-2 stimulates the differentiation of isotype switched memory B-cells into plasma cells although IgM cells represent the response of a CD27- population.

### 3. CPS and TT specific memory B-cells are identified in the circulation following vaccination although only the TT specific B-cells are long-lived.



**Figure 3: Vaccine specific IgG secreting memory B-cells identified by culture with SAC and IL-2 following conjugate vaccination.** Although memory B-cells were induced by both antigens, prior to booster vaccination significantly more TT specific, than CPS specific memory B-cells were identified. This is despite no individual having had a more recent TT vaccine and suggests that the TT memory B-cells are better maintained than their CPS specific counterparts.

### 4. There is an increase in TT specific T-cell activation in the circulation following CPS-TT conjugate vaccination



**Figure 4: T-cell proliferation in response to TT and CPS prior to and between days 7 and 11 following CPS-TT conjugate vaccination.** A consistent increase in the proliferation of T-cells in response to TT was identified following vaccination. No proliferation occurred in response to CPS at any time point confirming the lack of independent T-cell activation by the antigen.

## CONCLUSION

Despite the conjugation of CPS to TT and the activation of TT specific T-cells, several characteristics of the response to the two antigens were distinct. These include the production of IgM to the CPS despite previous vaccination and the relatively poor maintenance of CPS specific memory B-cells. These differences may result from distinct roles of the T-cells in the immune response generated against the two antigens or fundamental differences in the responding B-cell populations that are not overcome by conjugation. These may have implications for the long term efficacy of these vaccines.