IL-17A and IL-22 production by *in vitro* paediatric adenoidal and blood lymphocyte cultures in response to pneumococcal vaccine candidates – a potential tool for measuring mucosal immunity

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INTRODUCTION

• Current polysaccharide-based pneumococcal vaccines have their limitations and the development of new protein-based vaccines, which may offer a broader level of protection, is critical. •Experimental studies in mice demonstrate that antibody-independent, Th17-mediated immunity plays an important role in mucosal protection against *Streptococcus pneumoniae* (1) and other bacterial species (2) (Figure 1). •There is also evidence of IL-17A production by human immune cells in response to pneumococcal antigens (1,3). •A means of assessing Th17 responses may therefore prove valuable in the development and evaluation of new vaccines, especially if such responses are predictive of vaccine effects on colonisation, either at the individual or population level.

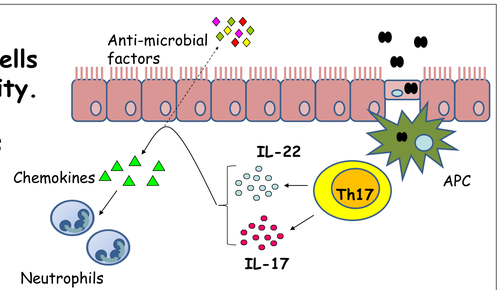

Figure 1. Th17 cells in mucosal immunity.

Invading pathogens are presented to CD4+ T cells which differentiate into IL-17 and IL-22 -secreting Th17 cells. These cytokines stimulate the epithelial cells to secrete neutrophil-recruiting chemokines, and anti-microbial factors.

METHODS

•Adenoidal or peripheral blood mononuclear cells (AMNC, PBMC) from 28 children (1-14 years) undergoing routine adenoidectomy were isolated using density gradient centrifugation. • Individual recombinant pneumococcal proteins (PspA, PsaA, CbpA or PhtD) or pneumococcal whole cell vaccine (WCV) were added to the mixed lymphocyte cultures (RPMI with 4mM L-glutamine, 10mM HEPES, 100U/ml penicillin, 10µg/ml streptomycin and 2% human AB serum) and left for 7-11 days at 37 C, 5% Co_2. • IL-17A and IL-22 were measured in the culture supernatants by ELISA (eBioscience Ltd, UK). • Nasopharyngeal swabs were taken from the children on the day of surgery to assess pneumococcal carriage. • Data groups were statistically analysed using Mann-Whitney U test.

RESULTS-1

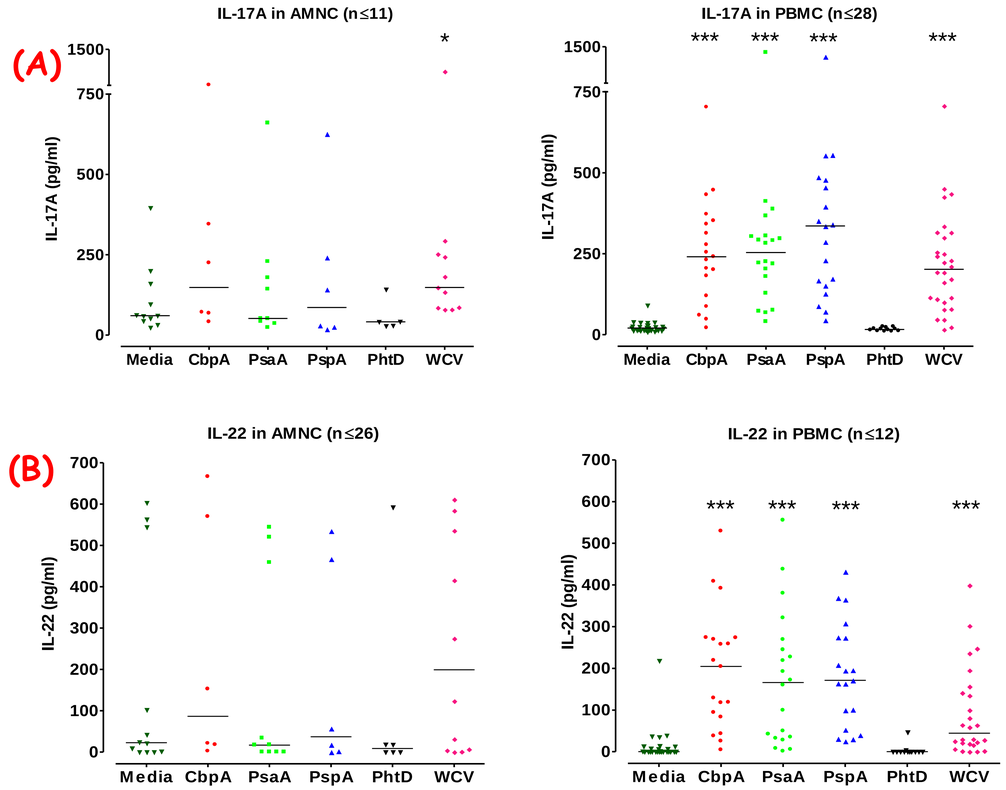

Figure 2. Generation of IL-17A (A) and IL-22 (B) in AMNC and PBMC in response to pneumococcal vaccine candidates. Data shown indicate individual responses with bar indicating group medians. Background response to media alone is also shown.

• IL-17A and IL-22 can be detected in both adenoids and peripheral blood from children, with higher responses detected in the blood. • IL-17A and IL-22 responses detected to CbpA, PsaA, PspA and WCV in PBMC were significantly greater than background responses (P<0.0001***)for all. • IL-17A generation by AMNC in response to WCV was significantly greater than background responses (p=0.03*). • There was little or no response to PhtD in any of our tests.

RESULTS-2

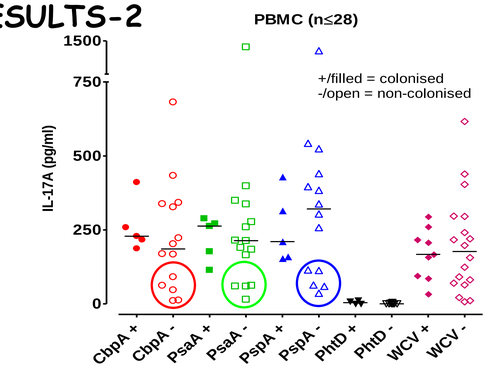

Figure 3. Generation of IL-17A in PBMC shown in relation to nasal carriage of *S.pneumoniae*.

• Approx. one third of the children were colonised with pneumococcus. • For CbpA, PsaA and PspA stimulations a small group of non-colonised children have low levels of IL-17A secretion (large circles), lower than those detected in any of the colonised children, although individual children in these groups differ depending on the antigen stimulation. • It is possible that this IL-17A readout may give an indication of the carriage histories of these children.

CONCLUSIONS

• Our results suggest a Th17 response is induced by pneumococcal WCV and recombinant antigens. •Greater cytokine responses are detected in PBMC than in AMNC which may be due an increased regulation of T-cell responses, as we have recently shown there are more T regulatory cells in adenoids than in blood (4) •There is a potential difference in the cell-mediated response according to the carriage status of the child and we will study children longitudinally to assess whether these peripheral blood responses can predict resistance to, or clearance of, carriage. • These studies provide a new insight into specific cell-mediated immunity to pneumococcal antigens in children and may represent a blood test that could be used to assess mucosal immune responses to candidate vaccines in future trials.

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ACKNOWLEDGEMENTS

Support for this project was provided by PATH