Th17 responses to pneumococcal protein antigens in children's nasal associated lymphoid tissue obtained by adenoidectomy could guide vaccine formulations

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Introduction

The search for protein based pneumococcal vaccines continues (1). One approach to reducing disease by *S. pneumoniae* is by reducing or preventing colonisation of the nasopharynx by the bacteria. Evidence published from murine studies has revealed antibody-independent, Th17 mediated immunity generated by pneumococcal whole cell antigen (WCA). Mice are protected by increased clearance of colonisation. The Th17 cytokine IL-17A has chemoattractant and neutrophil recruitment properties (1,2); illustrated in figure one below (3). A threshold level of IL-17A production predicts protection from colonisation by *S. pneumoniae* in mice (2). Understanding Th17 responses to vaccine candidate proteins in children could inicicate their potential to induce protective immunity and their suitability for use in vaccines.

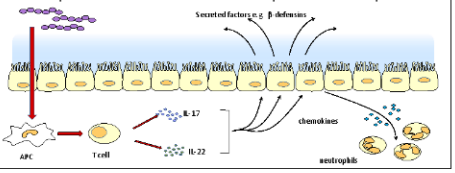


Figure 1 - Upon CD4+ T cell activation by an antigen presenting cell, CD4+ T cells have the ability to differentiate into Th17 cytokine secreting IL-17A and IL-22 cells which attract neutrophils to the site of infection, and stimulate the release of antimicrobial peptides (3).

Methods

• Using density gradient centrifugation mononuclear cells were extracted from adenoids and peripheral blood (AMNC, PBMC) from children aged 1-16 years undergoing routine acenoidectomy at Bristol Children's Hospital, UK.

• AMNCs or PBMCs were cultured in 48 well plates at $1x10^6$ cells/ml in RPMI with 4mM L-glutamine, 10mM HEPES, 100U/ml Penicillin, 10ug/ml Streptomycin and 10% foetal calf serum at 37°C/5% CO_2 for 7-11 days.

• Cultures were stimulated with each of several recombinant pneumococcal proteins, some well studied and others novel (4) and with an autolysin-negative acapsulate pneumococcal whole cell vaccine (WCV) expressing the non-cytolytic pneumolysin variant PdT.

• Supernatants were taken from the cultures and measured for IL-17A (day 7) and IL-22 (day 11) using eBioscience ELISA kits.

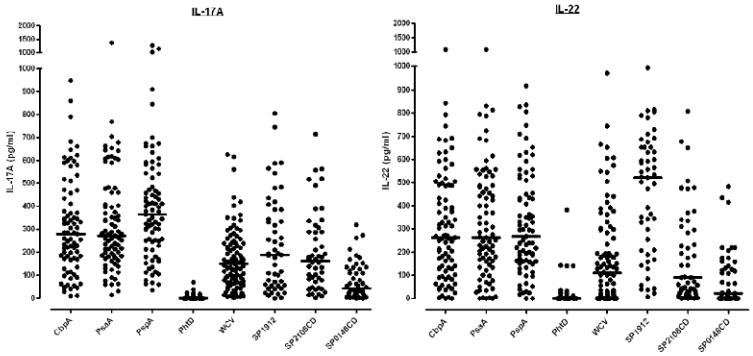
• Intracellular cytokine staining was carried out after the cells had been cultured for 7 days with antigen and restimulated on day 6 with antigen. The cells were incubated on day 7 for five hours with Golgistop, PMA and Ionomycin to enable cytokine detection before being surface stained with CD4, CD56 and TCRγδ antibodies, and intracellularly stained for IL-17A and IL-22.

•Colonisation with *Streptococcus pneumoniae* was determined by storing nasal swabs in STGG broth, before culturing the broth on a pneumococcal selective COBA plate for 24-48hrs. *S. pneumoniae* identification was confirmed by morphology, Optochin disc and a bile salts test.

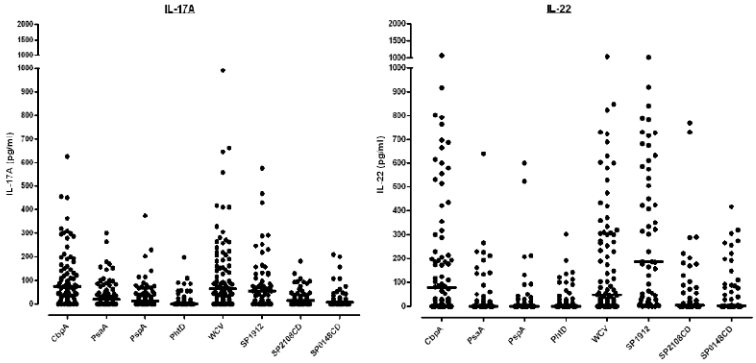
•Significance of differences between the responses to the antigens was assessed by analysis of variance (cut off $p<0.05$).

Results 1

A) PBMC



B) AMNC



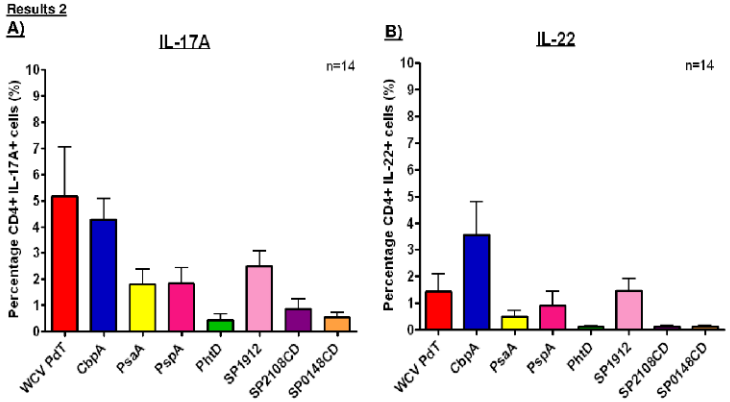
Results 1 – Detection of Th17 cytokines IL-17A and IL-22 in response to WCV and pneumococcal recombinant proteins in A) PBMC and B) AMNC

Data points represent individual children's cytokine levels detected by ELISA, and the bars represent medians. Numbers measured vary between 50 and 96 children due to the limitation of cell number obtained and the availability of antigens. Background has been subtracted from the values. Approximately 35% of children were detectably colonised with *S. pneumoniae* at time of surgery but previous colonisation histories were unknown. No consistent differences between culture positive and negative children were seen (data not shown). Differences in Th17-inducing properties of different antigens seen in PBMCs are less obvious in AMNC in which responses are generally lower. The antigen PhtD, which is in human vaccine trials, appears to be a poor inducer of human Th17 responses. Of the novel antigens tested, SP1912 incuced the highest Th17 responses on average. (ANOVA $p=<0.0001$)

Acknowledgements

We would like to thank all the children who took part, the staff at the Bristol Royal Children's Hospital, our nurses for recruiting the children to the study and to PATH for funding this work.

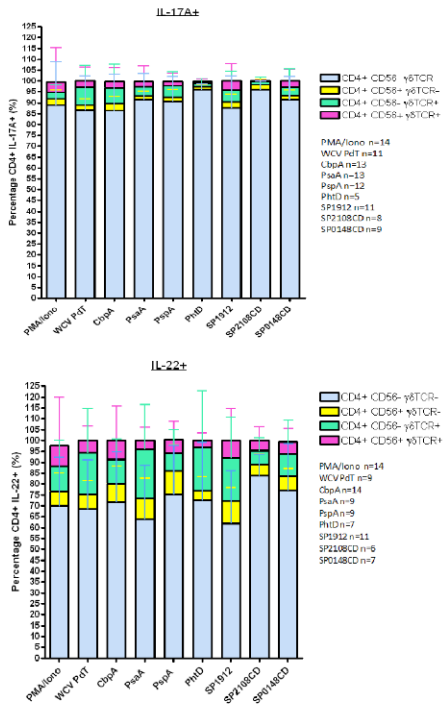
Results 2



Results 2 – Identification using intracellular cytokine staining of the percentage of CD4+ adenoid mononuclear cells induced by pneumococcal antigens to produce A) IL-17A and B) IL-22 at day 7 of culture

Using this approach to the evaluation of Th17 responses reconfirmed that significant differences exist between different pneumococcal antigens ($p=0.0002$ IL-17A, $p=0.0003$ IL-22) with the patterns suggested by immunoassay results being similar. Specifically, responses to PhtD are low and, among novel antigens, SP1912 induces both higher IL-17A and IL-22 responses.

Results 3



Results 3 – Identifying the cellular source of A) IL-17A and B) IL-22 in AMNC

In intracellular cytokine staining assays in AMNCs at day 7 the percentage of CD4+ Th17 cytokine-producing cells of the different CD56 and TCRγδ phenotypes shown induced by different pneumococcal antigens were counted. The bar represents the mean with the standard deviation shown.

In the case of IL-17A, the large majority of such cells were of the classic Th cell phenotype (CD4+ CD56- TCRγδ-).

However in the case of IL-22, a large minority of induced CD4+ IL-22 cytokine producing cells showed an innate phenotype, expressing either CD56, or TCRγδ or both.

Conclusions and future investigations

• Naturally-acquired Th17 responses to pneumococcal antigens vary widely between children. Such variation is to be expected and may reflect differences in age and immune maturity, differences in the number and timing of previous exposures to pneumococcus and differences in genetically acquired determinants of capacity to respond.

•Some antigens appear consistently to induce stronger or weaker Th17 cytokine responses than others.

•Patterns of response observed in cultures of PBMCs (systemic responses) are poorly predictive of responses seen in AMNCs (mucosal responses) both in incividuals and across the population.

• Although CD4+ T helper cells are clearly a source of Th17 cytokines induced by pneumococcal antigens in mixed cells cultures, other CD4+ cells with innate phenotypes also contribute, especially to production of IL-22.

•This approach to screening prospective vaccine antigens for their capacity to induce mucosal Th17 responses may prove valuable in predicting their capacity to impact on colonisation and transmission. However this can only be confirmed by human vaccine studies which evaluate impact on colonisation in detail (including measures of duration and density of colonisation)

References

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