

Session: OS197 Fruits hanging high or low? Novel vaccines update

**Category: 10e. Antibacterial vaccines**

25 April 2017, 14:18 - 14:28  
OS0982

## Immunogenicity of B-cell epitopes within virulent surface proteins of *S. pneumoniae* and opsonophagocytic killing assay of their specific anti-peptide antibodies in a rabbit model

Theodora Papastamatiou<sup>\*1</sup>, John Routsias<sup>2</sup>, Maria Mavrouli<sup>3</sup>, Eleni Dotsika<sup>4</sup>, Vana Spoulou<sup>5</sup>, Athanassios Tsakris<sup>3</sup>

<sup>1</sup>*First Dept of Pediatrics Athens Medical School; "agia Sophia" Children's Hospital; Aghia Sophia Children's Hospital, Makka Immunobiology Laboratory*

<sup>2</sup>*University of Athens School of Medicine; Department of Microbiology*

<sup>3</sup>*Medical School, University of Athens; Microbiology*

<sup>4</sup>*Laboratory of Cellular Immunology, Hellenic Pasteur Institute, Athens, Greece*

<sup>5</sup>*National and Kapodistrian University of Athens; "aghia Sophia" Children's Hospital; First Department of Pediatrics*

**Background:** Characterization of the fine specificity of antibodies against PhtD and PhtE virulent pneumococcal proteins revealed that they target epitopes within the common zinc-binding domain of Phts. We demonstrated that human anti-PhtE\_pep40 purified antibodies from sera of children with Invasive Pneumococcal Disease(IPD) could kill pneumococcus more efficiently than anti-PhtD\_pep19 in an Opsonophagocytic Killing Assay(OPKA). Murine vaccination with these epitopes showed that sera from mice immunized with PhtD\_pep19 could cross-recognize peptide PhtE\_pep40. Heterologous inhibition experiments also revealed that antibodies raised against peptide PhtD\_pep19 could be inhibited by peptide PhtE\_pep40. In this study we aimed to further evaluate the immunogenicity of these epitopes in rabbit immunization experiments.

**Material/methods:** According to our vaccination schedule New Zealand rabbits were primed subcutaneously in two rabbit groups with 500µg of PhtD\_pep19 and PhtE\_pep40 peptides, respectively, emulsified in complete Freund's Adjuvant (CFA). All rabbits were boosted on day 14 and 28 with 250µg of their relevant peptide emulsified in Incomplete Freund's Adjuvant (IFA). Serum samples were obtained before the immunization and then every two weeks. The kinetics of anti-peptide antibody concentrations and avidity were evaluated by ELISA assays, whereas we evaluated the functional capacity of anti-peptide rabbit antibodies against clinical isolated pneumococcal serotypes(PS)1, 3 and 19A by an OPKA.

**Results:** All immunized animals produced high levels of peptide-specific IgG antibodies compared to preimmune sera. We also found that, from the first to the last bleeding, immunized rabbits produced gradually higher antibody titer ( $p < 0.0001$ ). The Avidity Index(AI) of produced anti-peptide antibodies increased in parallel with the booster injections, as well as, a high AI of produced rabbit antibodies, targeting PhtD\_pep19 and PhtE\_pep40 peptides was detected ( $AI > 0.80$  and  $0.60$ , respectively). Rabbits immunized with peptides PhtD\_pep19 and PhtE\_pep40 had higher OPKA titers than the preimmune rabbit sera, respectively, against PS 1,3 and 19A( $p < 0.001$ ), whereas sera from rabbits immunized with PhtD\_pep19 exhibited a higher OPKA titer than PhtE\_pep40( $p < 0.05$ ).

**Conclusions:** The selected peptides demonstrated that they are capable of inducing a robust antigen-specific humoral response in immunized rabbits and their progressive avidity maturation could also be considered as evidence for peptide-specific T-cell activation and T-cell help. Additionally, immunization of rabbits with these epitopes verified, *in vitro*, the functional capacity of produced anti-peptide-antibodies against *S.pneumoniae*. Of note, anti-PhtE\_pep40 rabbit antibodies exhibited a lesser OPKA titer than anti-PhtD\_pep19. In line with our previous cross reactivity and heterologous inhibition assays in mice, it could be hypothesized that the enhanced functional activity of purified human anti-PhtE\_pep40 antibodies may be due to the potential of PhtE\_pep40 peptide to co-purify both anti-PhtE\_pep40 and anti-PhtD\_pep19 antibodies from serum of children recovering from IPD. Further experiments should be performed to explore the protective potential of peptides in order to be used as vaccine candidates.