Antigen Specific B Cells in the Immune Response to *Haemophilus influenzae* type b PRP Conjugate Vaccine

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SUMMARY

*Haemophillus influenzae* is a pathogenic gram negative bacterium, which exists in encapsulated and non-encapsulated forms. The serotype b of the encapsulated form can cause invasive infections such as epiglottitis, meningitis, septic arthritis, and septicemia in humans.

The protective immune response to *Haemophillus influenzae* type b (Hib) results from antibodies developed against the polyribosylribitol phosphate (PRP) capsular polysaccharide of the bacterium. Polysaccharide antigens are less immunogenic than protein antigens. In early childhood the immune response to polysaccharides is even more restricted compared to adults, due to the immaturity of the immune system. Children under two years are more susceptible to invasive Hib infections, as they are unable to mount an adequate antibody response against the PRP polysaccharide. To prevent invasive Hib infections in early childhood, infants in most developed countries are immunised against Hib with PRP conjugate vaccines as part of the routine immunisation schedule. Effective Hib vaccines have been developed by conjugating PRP with a carrier protein to elicit a strong response which has some characteristics of T-dependent antigen responses.

However, the details of the development of the antibody response to Hib conjugate vaccines are not fully understood. The aim of this study was to develop potential methods to identify, enumerate and characterise PRP specific B cells in young children and in adults following immunisation with Hib conjugate vaccines and compare with antigen-specific B cell responding to a pure protein antigen, tetanus toxoid (TT).
Immunofluorescence staining followed by flow cytometry was performed on peripheral blood lymphocytes (PBL) from Hib conjugate vaccine and TT immunised adults. PRP binding specific B cells could not be detected in PBL of immunised adults by immunofluorescence staining and flow cytometry, although TT binding specific B cells were identified, suggesting that PRP binding specific B cells may be present in very small numbers in peripheral blood or may be relatively confined to lymphoid tissues.

As an alternative, immunofluorescence staining procedure, murine anti-PRP monoclonal antibodies were developed for use in indirect immunofluorescence assays in an attempt to detect PRP specific B cells by flow cytometry. The anti-PRP monoclonal antibodies were of IgM class and had functional activity against Hib. However, the monoclonal antibodies proved not to be useful for isolating PRP binding specific B cells due to binding to lymphocytes through non-PRP molecules.

To test the hypothesis that PRP specific B cells may be relatively confined to secondary lymphoid tissue, flow cytometry and enzyme linked immune spot (ELISPOT) assays were developed and performed on PRP-T immunised (PRP conjugated to tetanus toxoid) mouse spleen and peripheral blood cells. Significant numbers of anti-PRP and anti-TT antibody secreting cells were identified by ELISPOT assays in spleen cells of mice after booster immunisation. The percentage of anti-TT antibody secreting cells identified was nearly twice the percentage of PRP antibody secreting cells. Very few anti-TT antibody secreting cells were identified in peripheral blood of immunised mice and the number of anti-PRP antibody secreting cells was extremely low in peripheral blood samples from these mice. In contrast to the ELISPOT results, PRP and TT binding specific B cells were not able to be identified by flow cytometry using spleen cells from immunised and boosted mice.
ELISPOT assays were performed on tonsil cells of children less than four years of age, who were immunised with Hib conjugate vaccines and TT vaccines as part of routine immunisation schedules, to identify anti-PRP antibody secreting cells. Anti-PRP and anti-TT antibody secreting cells were identified by ELISPOT assays in tonsil cells. The percentage of TT antibody secreting tonsil cells was two and a half times greater than that of PRP antibody secreting tonsil cells.

These results demonstrate that compared to TT specific B cells, PRP specific B cells are either not present in peripheral blood or circulate in very low numbers after immunisation with Hib conjugate vaccine. Furthermore, the generation of anti-PRP antibody secreting cells may be low in comparison to the generation of anti-TT antibody secreting cells, and the cells that are present may be relatively restricted to lymphoid tissues. In conjunction with previous studies, these findings further support the concept that Hib conjugate vaccines does not ensure a full TC response in spite of being coupled to a carrier protein. Since isolation of PRP antigen specific B cells using the techniques has proven to be a challenging task an alternative approach is proposed for characterization of B cells involved in antibody response to Hib conjugate vaccines.