Factors affecting the immunogenicity and protective efficacy of routine childhood immunisations

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CHAPTER 7. DEFINITIVE PROTECTIVE EFFICACY EXPERIMENTS: LUNG HISTOPATHOLOGY AND BRONCHOALVEOLAR LAVAGE FLUID ANALYSIS

Abstract

This thesis has examined the effect of adverse storage on the immunogenicity of pertussis, diphtheria and tetanus vaccines, the protective efficacy of pertussis vaccines and the effect of premature birth on antibody responses to routine childhood immunisations.

Methods

Murine Immunogenicity studies

Female Swiss outbred and Balb/c mice eight weeks of age were immunised intraperitoneally with whole cell pertussis vaccine (DTPw), acellular pertussis vaccine (DTPa) or normal saline on day 0 of each experiment. Blood was collected to determine IgG antibody responses to pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) on days 0 and 28. Vaccines were stored under ideal conditions (2^0C to 8^0C) or at -3^0C for 24 hours prior to immunisation for both strains of mice and also at -3^0C for 14 days or at -6^0C for 14 days for Swiss outbred mice.

Murine Protective efficacy studies

The murine model was extended to include a second immunisation at 28 days, and an intranasal challenge of live pertussis organisms two weeks after the second immunisation, using Balb/c mice. Vaccines were stored under the same conditions as for the immunogenicity studies, and also at -6^0C for 24 hours. At serial time points after challenge mice were sacrificed to determine lung clearance of organisms and IgG antibody responses to PT, FHA, PRN and diphtheria and tetanus toxoids. Bronchoalveolar lavages (BAL) for cell count and cytokine measurement and preparation of lungs for histopathological analysis were also performed in subsets of mice.

Human studies

Two studies were undertaken. In the first study, term and premature infants were recruited from two months of age to participate in a longitudinal study to determine IgG antibody responses to DTPa and Hib vaccine antigens before and after primary and 18 month booster immunisations. Comparisons were made between both term and premature infants and term infants and premature infant subgroups determined on the basis of gestational age at birth (extremely premature infants: gestational age at birth = 27 weeks, very premature infants: 28-32 weeks, premature infants 33-36 weeks).

In the second study, stored sera from premature and term infants enrolled in a previous study were used to perform longitudinal comparisons of the effect of immunising with DTPa or DTPw in the primary series and DTPa at 18 months on antibody responses to bacterial vaccine antigens. Cross sectional analyses were performed to confirm 18 and 19 month antibody concentrations in premature infants with different primary DTP immunisation schedules using the stored sera and sera from a second cohort of premature infants newly recruited at 18 months. In addition, further cross-sectional analyses were performed, by combining the two newly recruited premature infant cohorts and comparing term and premature infant and term and premature infant subgroup antibody responses to vaccine antigens at 18 and 19 months.

Results

Murine immunogenicity studies

In Swiss outbred mice, storage at -3^0C for 24 hours significantly reduced vaccine immunogenicity in all cases except to PRN in DTPa. Other adverse vaccine storage conditions either had no effect on, or paradoxically produced higher antibody concentrations than ideally stored vaccines. Antibody responses to DTPa and DTPw in

Swiss outbred mice were higher than those in Balb/c mice. In both mouse strains, antibody responses to DTPa were greater, but more variable, than those to DTPw.

Murine protective efficacy studies

In general, adverse vaccine storage did not alter lung clearance rates of *B. pertussis* or murine IgG antibody responses to vaccine antigens in DTPa or DTPw or serological responses to immunisation. However, mice immunised with DTPw did not develop antibody responses to PT and demonstrated greater concentrations of diphtheria and tetanus antibodies and lesser concentrations of pertussis antibodies than mice immunised with DTPa. Mice immunised with normal saline did not demonstrate pertussis responses until 14 days after infection and these were of a very small magnitude. No significant correlations between serological responses and lung clearance were demonstrated in these mice when analyses were performed by vaccine and storage condition. However, some of the comparisons tended towards significance with moderately negative correlations demonstrated. Hence, larger numbers of mice may be necessary achieve adequate power for these comparisons.

Immunisation with DTPa resulted in slightly more rapid lung bacterial clearance, less inflammation in stained lung sections, and fewer inflammatory cells in BAL fluid than mice immunised with DTPw or saline. Mice immunised with DTPa and normal saline demonstrated a Th-2 type cytokine response in BAL fluid, whereas immunisation with DTPw conferred a Th-1 cytokine profile. None of these parameters was affected by adverse vaccine storage.

Human studies

The general pattern of IgG antibody concentrations at different study time was consistent in both term and premature infants, with low level of maternal antibodies detected at 2 months, increased responses after primary immunisation measured at the 7 month study time, a decline in antibody concentrations at the 18 month study time with increased booster responses measured at the 19 month study time.

Premature infants demonstrated lower concentrations of IgG antibodies to all vaccine antigens throughout the cohort survey period. These were significantly lower than term infants in response to pertussis vaccine antigens at all study times with one exception (PRN at 19 months). In contrast, premature infant responses to diphtheria and tetanus toxoids and Hib PRP were not, in general, significantly lower than those of term infants. Extremely premature infants demonstrated the lowest antibody concentrations, and the magnitude of antibody responses in premature infants increased with increasing gestational age at birth.

Premature immunised with DTPa from two months of age in general demonstrated significantly higher antibody responses to pertussis antigens after primary and 18 month booster immunisations, but significantly lower responses to diphtheria and tetanus toxoids than premature infants immunised with DTPw in the primary series and with DTPa at 18 months regardless of gestational age at birth. Term infant comparisons were similar, at 7 months, but there were no statistical differences in PT, FHA or diphtheria antibody concentrations between the two groups of term infants at 19 months.

Combining the two newly recruited premature infant cohorts confirmed the term and premature infant comparisons of the first study with regard to PT, FHA, PRN and Hib PRP

antibody concentrations at 18 and 19 months. However, comparisons between term infants and premature infant subgroups were altered: very premature infant pertussis antibody concentrations became more comparable with those of term infants. Extremely premature infant pertussis antibody concentrations remained significantly lower than those of term infants. Diphtheria and tetanus antibody concentrations at 18 and 19 months were affected unpredictably by the addition of a second cohort of premature infants in the analyses.

Conclusions

Although adverse storage does not appear to diminish vaccine immunogenicity or protective efficacy in the short term, vaccines should be stored according to the manufacturers instructions until long term efficacy studies can be performed.

Premature infants demonstrated lower IgG antibody concentrations to all vaccine antigens, but were able to mount protective antibody responses to diphtheria and tetanus toxoids and Hib PRP. Premature infant responses to pertussis vaccine antigens were, in general, significantly lower than those of term infants at all study times, despite evidence of increased antibody concentrations after primary and 18 month booster immunisations. Extremely premature infants demonstrated the lowest antibody concentrations to all vaccine antigens, and in particular to pertussis vaccine antigens. There is no single serological correlate of protection against pertussis infection, however, and antibody avidity and cell-mediated immune responses were not examined in these infants. Therefore these parameters should be explored before changes are made to the immunisation schedule of premature infants.