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RESPONSE OF IgG SUBCLASS DEFICIENT PATIENTS TO H. INFLUENZA TYPE b POLYRIBOSE PHOSPHATE (PRP) DIPHTHERIA TOXOID CONJUGATE (PRP-D) VACCINE. Howard M. Rosenblatt, John M. Zahradnik, and Patrick H. McVerry (Spon. by William T. Shearer) Baylor College of Medicine, Texas Children's Hospital, Depts. of Pediatrics and Microbiology, Houston, TX, and Connaught Labs., Swiftwater, PA.

The efficacy of PRP-D vaccine in young infants has been documented in previous studies. We now report the response of patients with IgG subclass deficiency (GSD) to a single immunization with PRP-D. Patients with GSD ranging in age from 6 to 10 years were given a single i.m. dose of 20 µg PRP-D. Total serum anti-PRP antibody levels were determined using a modified Farr assay and radiolabeled PRP. Four patients with GSD and no major abnormalities in B- and T-cell subsets or in vitro mitogen stimulation had a mean pre-immunization anti-PRP level of 1.61 µg/ml which rose to 22.6 µg/ml 3-4 weeks after immunization. The mean increase in anti-PRP for 3 patients with IgG2 deficiency was 18.8 µg/ml. Two patients with IgA and IgG2 deficiency responded with post-immunization anti-PRP levels of 10.8 and 40 µg/ml. Both of these patients failed to respond to immunization with a polyvalent pneumococcal polysaccharide (PS) vaccine. One patient with isolated IgG3 deficiency responded normally to diphtheria and tetanus toxoid, to 3 of 4 pneumococcal serotypes tested, and had a rise of anti-PRP antibody from 0.6 to 28.4 µg/ml. A fifth patient with common variable immunodeficiency had no response to immunization with any of the above vaccines. These data suggest that protein conjugate vaccines may lead to effective immunization of patients with defective PS antigen processing.

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THE ROLE OF IgA₁ RHEUMATOID FACTOR (RF) IN THE FORMATION OF IgA₁-CONTAINING CIRCULATING IMMUNE COMPLEXES (CIC) IN HENOCH-SCHONLEIN PURPURA (HSP). Frank T. Saulsbury. (Spon. by Robert L. Chevalier), University of Virginia Medical Center, Department of Pediatrics, Charlottesville, VA.

Current evidence suggests that CIC are involved in the pathogenesis of HSP, and several studies have demonstrated both IgA and IgG containing CIC in patients with HSP. Recent work from this laboratory showed that over 50% of children with HSP have IgA RF. The present study was performed to investigate the role of IgA RF in the formation of IgA containing CIC in HSP, and to determine the subclass composition of IgA RF. CIC were isolated from the sera of 22 children with HSP and 13 controls by means of polyethylene glycol (PEG) precipitation. The percentage of IgG, IgA, and IgM precipitated by PEG was significantly greater in HSP patients than controls (P<0.01). There was a strong correlation (r=.723, P<0.001) between the amount of IgG and IgA in the PEG precipitates from HSP patients, but not controls. HSP patients had significantly higher levels of IgA RF in their serum (P<0.05) and in their PEG precipitates (P<0.05) compared to controls. PEG precipitation eliminated IgA RF activity from the serum of seven of eight HSP patients tested; IgA RF was recovered in the PEG precipitates from all patients. Testing of HSP patients showed that IgA₁ was the predominant IgA subclass of the serum IgA RF (P<0.02) and PEG precipitate IgA RF (P<0.01). These results suggest that IgA RF is a constituent of IgA CIC in HSP, and that IgA RF is predominantly IgA₁ in composition.

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TRANSFUSION-ACQUIRED HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION IN NEONATES. Frank T. Saulsbury, Randolph F. Wykoff, and Robert J. Boyle. (Spon. by Robert L. Chevalier), University of Virginia Medical Center, Department of Pediatrics, Charlottesville, VA.

Eleven neonates received blood from two HIV infected donors. All developed laboratory and/or clinical evidence of HIV infection, usually in the first year of life. Nine of 11 had serum antibody to HIV when tested between 9 and 16 months of age; two seronegative infants were severely hypogammaglobulinemic when tested. Nine patients developed a variety of illnesses characterized by hepatosplenomegaly, lymphadenopathy, chronic diarrhea, failure to thrive, and thrombocytopenia. Infections, including pneumonia, mucocutaneous candidiasis, and sepsis were a major source of morbidity and mortality. Two children have remained continuously asymptomatic. In follow-up ranging from two to four years, five patients have died, four others had HIV associated illnesses, but recovered and are now healthy. All patients had immunologic abnormalities; the most consistent finding was a decreased proportion of T-helper cells. Three patients had panhypogammaglobulinemia. These infants had significantly lower numbers of T-helper cells compared to patients with normal or increased serum immunoglobulin concentrations (P=0.012). We conclude that exposure to HIV via transfusion in the neonatal period results in an extremely high rate of infection with substantial mortality and morbidity, but clinical recovery occurs in some patients. Second, hypogammaglobulinemia may be more common in infants with HIV infection than previously appreciated.

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ONTOGENY OF THE SUBCLASS OF ANTIBODY TO PNEUMOCOCCAL POLYSACCHARIDE TYPE 3. Desmond Schatz, Douglas J. Barrett, University of Florida College of Medicine, Department of Pediatrics, Gainesville, Florida

Human antibodies to bacterial polysaccharides consist primarily of IgG and are largely restricted to the IgG2 subclass. We examined the ontogeny of the IgG subclass response to pneumococcal polysaccharide type 3 to determine if the poor response of infants to immunization with polysaccharide antigens is due to a diminished capacity to form this subclass of antibodies. Sera from 33 patients aged 2 months to 25 years who had previously been shown to respond to polyvalent pneumococcal polysaccharide vaccine with IgG antibodies (greater than twofold rise in post-immunization titer) were assayed by ELISA using monoclonal antibodies specific for IgG1, IgG2, IgG3, or IgG4. IgG1 antibodies to pneumococcal polysaccharide type 3 were uniformly low in all age groups. In contrast, IgG2 antibody activity was lowest in children less than the age of 2 years (170±20 ng/ml), but rose progressively in the age group 2-5 years (210±40 ng/ml), 5-10 years (330±30 ng/ml), and over the age of 10 (390±30 ng/ml) (differences significant at p<0.005 by ANOVA). Since the prevention of infection using a purified bacterial polysaccharide vaccine appears to depend on an adequate serum level of IgG2 antibody, the delay in the ontogeny of the IgG2 subclass explains the relative ineffectiveness of purified polysaccharide vaccines in children under the age of 2 years. Our findings are consistent with the hypothesis that purified bacterial capsular polysaccharide antigens preferentially activate IgG2-committed B cell clones at all ages.

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HUMAN IMMUNIZATION IN VITRO. Victoria Schauf, Ken Bengtson, Pat Holobaugh Nassau Cty. Med. Ctr., SUNY Stony Brook Health Sci. Ctr., Dept. of Ped., East Meadow, NY.

In vitro sensitization of human peripheral blood mononuclear cells is potentially useful in evaluation of immune responses, for hybridoma production, and for immunogenetic and vaccine research. Secondary (2^o) in vitro lymphoproliferative responses are obtained after in vivo sensitization. However, primary (1^o) in vitro immunization with soluble antigens has been more difficult. We have now induced 1^o and 2^o responses in vitro for purified antigens, complex soluble antigens, a lipid antigen presented in liposomes, and bacteria. PPMC were incubated at 10⁶/ml in flasks for 9-11 d with a 5 log concentration range of BSA, KLH, PPD, coccidioidin, liposomes with M. leprae phenolic glycolipid 1 (PG-1-L), or irradiated M. leprae. After washing, cells in microtiter plates were restimulated with antigen or fed without antigen. After 48 h cells were pulsed with ³H-thymidine. Results are shown as ratio CPM with restimulation:CPM without restimulation. Ratios ≥ 1.9 reflect lymphoproliferative responses; ratios ≤ 0.3 indicate suppression of responses (both p < .05). Sensitization was designated as 1^o if prior reactivity by skin test and/or lymphocyte transformation was absent, or 2^o if present. Responses were 1^o for BSA (maximum ratio = 2.8), KLH (2.7), PPD (10.6), coccidioidin (2.5), and PG-1-L (6.3); 2^o responses occurred for KLH, PPD, and M. leprae. 1^o suppression was seen for BSA, coccidioidin, and PPD. The technique may be useful in inducing immune responses without exposing the subject.

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TUFTSIN MONITORING OF SPLENIC FUNCTION POST NEONATAL SPLENECTOMY. Michael S. Schimmel, Zvi Spierer, Itzhak Vinograd, Arthur I. Eidelman. Shaare Zedek Medical Center, Department of Neonatology, Rokach Hospital, Department of Pediatrics and Division of Pediatric Immunology, Jerusalem, Tel Aviv, Israel.

Tuftsins, one of the proposed parameters of splenic function, was utilized as a monitor of splenic immunologic activity in a patient who underwent splenectomy and autologous spleen implantation at age of 30 hours after decapsulation and massive hemorrhage. Serial tuftsins levels were obtained at ages 3, 4 1/2, 6, 13 and 26 months. Tuftsins levels were measured by ELISA technique, normal levels are above 200 ng/ml. Tuftsins levels were below 100 ng/ml at the age of 3 and 4 1/2 months, between 100 and 200 ng/ml at 6 months and repeatedly above 200 ng/ml from age 13 months. Normal tuftsins levels were measured only at age 13 months or more despite the fact that Technetium 99m scan demonstrated uptake at the age of 6 months and blood smears from age one week did not show any Howell Jolly bodies. Antibiotic prophylaxis was discontinued only after tuftsins levels became normal. Given previous reports of increased infection risk even in patients with documented splenic tissue post autologous implantation, this case suggests that tuftsins levels can be utilized as a more specific indicator of splenic activity and as a guide for discontinuation of antibiotic prophylaxis.