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DISTRIBUTION AND FUNCTIONAL ACTIVITY OF HUMAN IgG SUBCLASS-SPECIFIC ANTIBODIES TO NATIVE TYPE III GROUP B STREPTOCOCCAL (III-GBS) POLYSACCHARIDE.

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The human immune response to carbohydrates is believed to be restricted primarily to the IgG₂ subclass which, relative to IgG₁ and IgG₃, has poor complement (C) fixing activity. However, for bacterial polysaccharides, the immunospecific IgG subclass distribution and its functional correlates are poorly defined. Following immunization with III-GBS vaccine, serum from a healthy adult was subjected to ion exchange chromatography yielding 1935mg/dl IgG and 1200µg/ml III-GBS antibody (Ab). Employing agammaglobulinemic serum as a C source, bactericidal activity (BA) >1 log₁₀ for log phase III-GBS was present to a 1:400 dilution of the IgG. After protein A-Sepharose (PAS) chromatography, 72% of IgG₃ but <1% each of IgG₁, 2 and 4 were recovered as detected by IgG subclass-specific ELISA. The IgG₃ had no detectable III-GBS Ab or BA. The fraction then eluted from PAS had 47% of the initial IgG, 48% of III-GBS Ab and achieved >1 log₁₀ BA at a 1:200 dilution. Digestion of IgG with papain for 4 hr at 37°C (to selectively cleave IgG₁ but not IgG₂) followed by Sephacryl chromatography yielded an intact IgG pool containing 31% of initial IgG, 69% of III-GBS Ab, 58% of IgG₂, but only 17% of IgG₁. This IgG₂-rich pool had BA >1 log₁₀ to a 1:400 dilution. These results indicate that IgG₃ has no functional activity for III-GBS. In contrast, IgG₂ comprises a disproportionately high percentage of III-GBS immunospecific Ab and appears to be the major heat-stable opsonin in adult sera.

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IMMUNO-ADJUVANT EFFECT OF DTP, DT, AND T WHEN COMBINED WITH HAEMOPHILUS INFLUENZAE TYPE B (HIB) POLYSACCHARIDE VACCINE. Mary P. Glode, Neal A. Halsey, Jeffrey A. Solomon. The Children's Hospital and University of Colorado School of Medicine, Denver, CO and Tulane University School of Medicine, New Orleans, LA.

Infant rats were immunized subcutaneously on days 5, 10 and 15 of age with a single lot of HIB vaccine. Lyophilized vials of HIB vaccine were reconstituted with either phosphate-buffered saline (PBS), diphtheria-tetanus toxoid and pertussis vaccine (DTP), diphtheria-tetanus toxoid (DT), or tetanus toxoid (T). At 20 days of age, animals were challenged intranasally with live HIB organisms and blood cultures were obtained 48 and 96 hours post-challenge. The following rates of bacteremia were observed.

Vaccine	Number Bacteremic/Total (%)
DT (Lederle)	21/24 (87)
DTP (Lederle)	108/138 (78)
HIB109+PBS	22/35 (63)
HIB109+DTP (Lederle)	14/49 (28)
HIB109+DT (Lederle)	12/56 (21)
HIB109+DT (Wyeth)	10/20 (50)
HIB109+T (Lederle)	4/22 (18)

HIB109+DTP was significantly more effective than HIB109+PBS in preventing HIB bacteremia after challenge (p=.003). Although pertussis vaccine has been assumed to be responsible for the adjuvant effect, our results indicate that Lederle DT vaccine alone exerts an adjuvant effect (p=.0002). HIB109+T was also significantly more effective than HIB109+PBS in preventing bacteremia (p=.002). These findings may have important implications for HIB vaccine testing in human infants.

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PROTECTIVE LEVEL OF HUMAN IgG ANTIBODY TO THE TYPE SPECIFIC POLYSACCHARIDE OF GROUP B STREPTOCOCCUS (GBS) TYPE III. Samuel P. Gotoff, Christine Odell, Cynthia K. Papierniak, Melvin E. Klegerman, Kenneth M. Boyer, Pritzker Sch. of Med., Univ. of Chicago, Dept. of Pediatrics, Michael Reese Hosp., Chicago, Ill.

We determined the serum concentration of human IgG antibody against native capsular polysaccharide of GBS type III which completely protected passively immunized 21 day old Swiss/Cox mice against lethal IP challenge with four clinical strains of GBS type III. Antibody was measured by ELISA, standardized by quantitative precipitation analysis of an affinity-chromatographed IgG fraction and corrected for non-precipitating antibody. The concentration of antibody in pools of mouse sera 24 hr after IM administration was proportional to the amount injected. A level of 1.3 µg/ml protected 97% (126/130) of mice from challenges between LD 80-96. Antibody to GBS type III did not provide protection from challenge with GBS types Ia and II.

Antibody to GBS type III ranged from <.02 to 21.7 µg/ml in sera of 102 pregnant women (median .05 µg/ml). 20% had concentrations >1.3 µg/ml. Levels in 25 colonized women ranged from 0.1 to 10.7 µg/ml (median 0.78 µg/ml, p<.001). Concentrations of antibody in sera from 42 infected infants were <0.3 µg/ml. Transplacental passage of antibody was determined for 30 pregnancies where maternal concentrations were >1 µg/ml. The relationship of the ratio of cord/maternal levels to gestational age was linear (r=0.758) and protective levels were demonstrated in infants with gestational ages between 28 and 36 weeks. The experimentally derived protective level is consistent with clinical observations and provides a useful guideline for the assessment of immunity.

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FURTHER STUDIES OF THE RESPONSES TO HAEMOPHILUS INFLUENZAE TYPE b VACCINE IN RELATION TO THE KM(1) AND GM(23) Ig ALLOTYPES. Dan M. Granoff, Eyla G. Boies, and Janardan Pandey, Washington University School of Medicine, St. Louis, and Med. Univ. S. Carolina, Charleston.

In experimental animals, there are genes associated with Ig allotypes which regulate immune responses. We examined the responses of children immunized with Haemophilus influenzae type b polysaccharide vaccine (PRP). 97 children, ages 12 to 83 mos (M=36.8±19), were immunized. Sera were obtained prior to immunization and 2 mos later. Total antiPRP was measured by a radioantigen-binding assay. IgG antiPRP was measured by ELISA. AntiPRP responses were lower in blacks lacking the Km(1) Ig allotype.

Results in Blacks		AntiPRP Antibody		
		Mean Age (Mos)	Farr (GM, ng/ml)	ELISA (GM, Recip. Titer)
Km(1)	No.	Pre	Post	Post
Pos.	34	31	419	3083
Neg.	12	32	294	871

In whites, there were no significant differences in antibody response in relation to the Km(1) or Gm(23) allotypes. In conclusion, blacks with Km(1) but not whites have higher IgG responses to PRP vaccine. Our findings corroborate previous results with a different Hib vaccine (PRP complexed to pertussis vaccine) (J.Clin.Invest. 74:1708, 1984). The present data support a genetic basis for immune response to the PS component of the vaccine. Further, the children found in our previous study to be genetically at greatest risk of Hib disease [Blacks lacking Km(1)] are the group least likely to benefit from immunization.

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IgG1 ANTIBODY RESPONSES OF CHILDREN AND ADULTS IMMUNIZED WITH HAEMOPHILUS INFLUENZAE TYPE b (Hib) POLYSACCHARIDE VACCINE (PRP). Dan M. Granoff, Penelope G. Shackelford, Eyla G. Boies, and Robert S. Munson, Jr., Wash. Univ. Sch. of Med., St. Louis.

IgG was purified by gel filtration of sera from 3 adults immunized with PRP. The ratio of IgG1 to IgG2 anti-PRP was determined in affinity purified anti-PRP. We also determined IgG1 anti-PRP by measuring anti-PRP radioantigen binding activity before and after column immunoabsorption of IgG employing a highly specific monoclonal prepared locally (HG-11). 38-46% of the post-vaccine serum IgG anti-PRP was of the IgG1 subclass. An ELISA was then used to measure IgG and IgG1 anti-PRP antibody in sera from 16 normal adults; and from 97 children 12 to 83 mos of age and 8 adults immunized with PRP. Only 2 of 16 unimmunized adults had IgG1 titers >1:49. The IgG1 anti-PRP antibody 2 mo after immunization was age-dependent.

Age (mos)	No. Tested	% with Titters >1:49	IgG1 Anti-PRP Geo. Mean (Recip)
12-23	33	15	33
24-47	39	26	44
48-83	25	52	109
Adults	8	100	282

12 of 35 children had high IgG antiPRP responses (post vaccine titers >1:400) but no detectable IgG1 antibody (IgG1 titer <1:49). Thus, IgG1 is an important component of the IgG anti-PRP response of some but not all immunized subjects. In addition, although serum IgG1 reaches adult concentrations in the first year of life, most children of this age cannot respond to immunization with IgG1 anti-PRP whereas many older children can.

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QUANTITATIVE MEASUREMENT OF C-REACTIVE PROTEIN (CRP) IN CEREBROSPINAL FLUID (CSF) OF PATIENTS WITH BACTERIAL MENINGITIS. Barry M. Gray, Don Simmons, Henry F. Mason, Scott K. Barnum, and John E. Volanakis. Depts. of Pediatrics and Medicine, Univ. of Alabama at Birmingham

The quantitation of CRP in CSF was investigated to aid in distinguishing bacterial from non-bacterial meningitis and other conditions. Previous work has been limited by the lack of a sensitive quantitative assay. This report describes results with a solid-phase sandwich RIA using rabbit anti-CRP coated polystyrene microwells and radiolabeled mouse monoclonal anti-CRP. CRP levels were measured in the CSF of 139 infants and children: 43 had culture-proven bacterial meningitis, 35 had aseptic CNS infections, and 61 had other conditions without CNS involvement. The mean CRP concentration in the bacterial group was 4900 ng/ml (range 0-51,100 ng/ml). This was significantly higher than for the aseptic group (mean 66, range 0-438 ng/ml) and others (mean 141, range 0-1750 ng/ml). Most patients with bacterial meningitis (31/43=72%) had levels >500 ng/ml, whereas those with aseptic (0/35) or other conditions (4/61=7%) were usually <500 ng/ml. In general, there was little correlation between CRP level and CSF protein, glucose, or neutrophil count, but some patients with bacterial meningitis apparently had a poor or delayed inflammatory response, accounting for their low CRP levels. These data suggest that measurement of CRP in CSF may be a useful, though not infallible, predictor of bacterial meningitis, provided that methods for CRP detection are sensitive to <500 ng/ml.