936 NORMAL MICROTUBULE (MT) ASSEMBLY BY CHEDIAK-HIGASHI (CH) PMNs. D. Anderson, B. Hughes, L. Wible, C.W. Smith & B.R. Brinkley. Baylor College of Medicine, Houston, TX & Michigan State University, E. Lansing, MI.

A new indirect immunofluorescent technique was employed in studies of a 1 y/o M with CHS to delineate possible functional relationships between assemblage of MTs & PMN motility, morphology & surface distributions of adhesion or lectin binding sites. CH PMNs exposed to gradients of fMLP demonstrated diminished orientation/migration ($p < .001$) but normal numbers of MT/PMNs [34±5 (CH), 36±7 (control)] & normal MT lengths - [CHS; 6.9±2.0 μm (PBS) → 10.1±3 μm (fMLP) vs control; 7.1±2 μm (PBS) → 10.9±3.1 μm (fMLP)]. MTs of CH & normal PMNs were equally susceptible to depolymerization by colchicine (μM) & their reassemblage following fMLP stimuli was normal. Shape change by CH PMNs stimulated in suspension with fMLP (2 nM, 5 min) was diminished [mean % bipolar + uropod forms = 30±7 (CHS), 92±6 (controls), $p < .001$]. Colchicine (μM) promoted significantly less bipolar shape change of CH PMNs (10±6%) as compared to normal PMNs (55±10%) ($p < .001$). Conditions promoting a redistribution of surface binding sites for albumin coated latex beads (ACLB) to the cell uropod in normal PMNs [colchicine (μM) or fMLP (0.1 nM, 10 nM)] failed to redistribute (cap) ACLB binding sites of CH PMNs. In contrast, CH PMNs demonstrated enhanced spontaneous concanavalin A capping [55±7% (CH), 8±6% (control), $p < .001$] or capping following colchicine (μM) preincubation [84±11% (CH), 61±10% (control)]. Thus, abnormalities of PMN mobility, shape change & surface distributions of lectin or ACLB binding sites mediated by chemotactic factors are unrelated to pathologic MT assembly.

937 ANTIBODY (Ab) TO THE CAPSULAR POLYSACCHARIDE (PRP) OF HAEMOPHILUS INFLUENZAE B IN 2 TO 6-MO-OLD INFANTS VACCINATED WITH PRP OLIGOSACCHARIDES CONJUGATED TO THE DIPHTHERIA PROTEIN CRM_{197}. Porter W. Anderson, Michael E. Pichichero, Univ Rochester, Dept. Peds, Roch NY (Spon. David H. Smith)

A reducing oligosaccharide preparation (vs) from PRP was conjugated by reductive amination to CRM_{197}, a non-toxic mutant protein of diphtheria toxin. This conjugate, Dcr-vs-1, was previously reported to elicit memory-type Ab responses to PRP in children 12-30 mo of age. In the present study 25-μg doses were given to healthy infants at ages 2,4, and 6 mo along with conventional DTP vaccine, in a separate site. There were no adverse reactions. Sera were taken before each injection and 1 mo after the third. The sequence is complete for 4 subjects. Anti-PRP Ab titers were determined by radioantigen binding:

	Anti-PRP Ab, μg/ml, at age			
Subject	2 mo	4 mo	6 mo	7 mo
KG	0.13	0.056	1.0	3.3
JH	<0.020	<0.020	1.2	4.2
MM	<0.020	<0.020	0.062	1.5
EC	0.41	0.092	0.020	<0.020

Thus no rises were seen after the 1°. There were distinct rises after the 2° and even larger rises after the 3° in 3 subjects. Subject EC, who had no detectable response, also had the highest pre-vaccination level (presumably maternal Ab). Additional infants and the Ig class specificity and bactericidal activity of the antibodies are being studied. The approach seems promising for prevention of H. influenzae b infection in the age range of greatest susceptibility.

938 AUTOIMMUNE PROFILE OF LYMPHOCYTE SUBPOPULATIONS IN AN ADOLESCENT WITH HISTOPLASMA CAPSULATUM (HC) MEDIASTINAL FIBROSIS (MF). Stephen C. Aronoff, Rebecca J. Turner, Kay Edmonds, Mary Ellen Kleinhenz, Steven W. Guyton and Jerrold J. Ellner (Spon. by William T. Speck). Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Departments of Pediatrics, Medicine, and Surgery, Cleveland, Ohio.

MF following HC infection is regarded as a hyperimmune phenomenon. Circulating lymphocyte subpopulations and lymphocyte blastogenesis were studied before (pre-Rx) and two months after surgery (post-Rx) to remove a mediastinal mass in a 14 y.o. male with HCMF. Pre-Rx, the OKT_4/OKT_8 ratio was 4.8 (normal <2.5) and decreased to 2.8 post-Rx mainly due to an increase in OKT_8-bearing lymphocytes from 12% to 19% (normal 12-24%). Immunoglobulin-bearing cells also increased post-Rx from 15% to 27% (normal 5-17%). OKT_3 reactive cells were unchanged. Streptokinase-Streptodornase (SK-SD) failed to induce blastogenesis in peripheral blood mononuclear cells (PBMC) pre-Rx. Depletion of adherent cells resulted in a positive response (stimulation index 5.6). SK-SD and HC mediated blastogenesis were observed in PBMC post-Rx (S.I. 8.1 and 4.5 respectively). Absent blastogenic responses in this form of HC infection appear to result from suppression by adherent cells. The pattern of circulating lymphocyte subpopulations noted pre-Rx is similar to classical autoimmune diseases supporting the hypothesis that the development of MF after HC infection is immune-mediated.

939 PROSTAGLANDIN SYNTHESIS BY RABBIT ALVEOLAR MACROPHAGES MEDIATED BY THE ALTERNATIVE COMPLEMENT PATHWAY ACTIVATION. Carlos M. Arroyave, Wei Hsueh, and Ruth L. Jordan. Northwestern University, Children's Memorial Hospital, Departments of Pediatrics and Pathology, Chicago Illinois, 60614.

Prostaglandin (PG) synthesis by rabbit alveolar macrophages (Mφ) was reported to be stimulated by heterologous , homologous and, autologous sera. In the present study we stimulated alveolar Mφ prelabelled with ^{14}C-arachidonic acid with various serum preparations, complement proteins and quantitated their PG release by thin layer chromatography. Our experiments showed that: 1) trypsinized rabbit serum(RS), RS activated with aggregated IgG and zymosan, stimulated PG release by Mφs. 2) Activation of RS with zymosan was more efficient stimulating PG synthesis than RS activated with aggregated IgG. 3) When C4 deficient guinea pig serum was used, it was clear that activation of the alternative pathway of complement (APC) independent of the classical pathway, stimulated PG release. 4) We were able to abolish the stimulatory effect of serum and activated C3 (but not zymosan) by blocking the Mφ C3b receptor (c3bR) with anti-C3bR preincubation. Thus we concluded that activation of the alternative pathway of complement plays an important role in PG synthesis by alveolar macrophages.

● **940** T-CELL INDEPENDENT HUMAN PNEUMOCOCCAL POLYSACCHARIDE ANTIBODY SYNTHESIS. Douglas J. Barrett and Elia M. Ayoub, Department of Pediatrics, University of Florida College of Medicine, Gainesville.

Antibody responses to Pneumococcal Polysaccharide type 3 (PnAb) are independent of T Cells in the murine model. To confirm the putative T cell independent nature of the response in humans, we studied PnAb secretion by in vitro lymphoctye cultures. Twelve day culture supernatants of blood mononuclear cells drawn before and 1 and 3 weeks post-immunization with Pneumococcal Vaccine were assayed for total IgG and PnAb by ELISA. Maximum PnAb secretion occurred at 1 week post-immunization in both unstimulated (177 ± 90 ng/ml) and pokeweed stimulated (300 ± 200 ng/ml) cultures. PnAb secretion was not due to polyclonal stimulation since no change in anti-tetanus toxoid secretion was found and a paradoxical decrease in total IgG secretion occurred (pre=2.4 mcg, 1 wk post = 1.3 mcg, $p < .001$). Addition of purified pneumococcal type 3 antigen (.001-10 ng/ml) had no effect on PnAb secretion. B cell enriched T cell depleted cultures had similar PnAb kinetics to unfractionated mononuclear cells: pre = 1.5 ± 1.6 ngAb/mcgIgG, 1 wk post = 180 ± 175 ngAB/mcgIgG, 3 wk post = 381 ± 380 ngAb/mcgIgG. Addition of T cells + pokeweed mitogen, purified IL-2, or allogeneic T helper factor from mixed lymphocyte culture supernatant had no effect on PnAb secretion expressed per mcg total IgG. These studies suggest that Pneumococcal Polysaccharide type 3 is a T cell independent antigen in humans which may activate B cells in a manner distinct from conventional protein antigens.

† **941** PNEUMOCOCCAL TYPE 3 ANTIBODY IS RESTRICTED TO THE IgG2 SUBCLASS. Douglas J. Barrett and Elia M. Ayoub, Department of Pediatrics, University of Florida College of Medicine, Gainesville.

To determine if human antibodies to Pneumococcal Polysaccharide type 3 are restricted in IgG subclass diversity, we tested sera from normal volunteers drawn before and after immunization (n=9). Subclass-specific ELISA techniques using murine monoclonal antibodies to human IgG1, IgG3, IgG4 and the Ig fraction of sheep anti-IgG2 were developed. Enzyme-conjugated antibodies were shown to be specific by testing against homologous and non-homologous IgG subclasses in direct ELISA using WHO myeloma reference standards. The relative sensitivity of each subclass antibody conjugate differed as shown by reaction in competitive binding ELISA where 50% inhibition of binding for IgG1=0.1 mcg, IgG2=1.75 mcg, IgG3=0.25 mcg and IgG4=0.175 mcg. End-point serum titer was defined as that dilution giving an optical density twice nonspecific background and was normalized to reflect the relative sensitivity of each subclass-specific assay. Pre-immunization geometric mean titers of serum antibody were IgG1= 13.5, IgG2=258.7, IgG3=7.0, and IgG4=3.5. Post-immunization titers were significantly higher for IgG2=955.0 ($p < .05$) but unchanged for IgG1=20.8, IgG3=6.6, and IgG4=4.0. Mean pre to post-immunization increases in antibody titer were significantly higher for IgG2=5.75 ($p < 0.05$) compared to IgG1=1.55, IgG3= 1.0, and IgG4=1.0. These results demonstrate that 90-95% of human antibody to the putative T-cell independent antigen Pneumococcal Polysaccharide type 3 is restricted to the IgG2 subclass.