866 PROTECTIVE AND OPSONIC ACTIVITY OF IgG SUBCLASS ANTIBODIES (Ab) AGAINST HAEMOPHILUS INFLUENZAE TYPE B (Hib). Joyce J. Schoettler, Kwang Sik Kim, Jane H. Hong, and Douglas C. Heiner, Harbor-UCLA Medical Center, Department of Pediatrics, Torrance, California, and Children's Hospital of Los Angeles, Department of Pediatrics, Los Angeles, California.

Ab to the capsular antigen of Hib is considered protective in humans, but the role of Ab of various immunoglobulin classes and subclasses is not well known. We evaluated the protective nature of sera from 13 children, 3 of whom were immunized with polyribosylribitol phosphate (PRP) and 4 with PRP conjugated to diphtheria toxoid (PRP-D); 6 were immunized with PRP conjugated to outer membrane protein of N. meningitidis. Each serum was used to passively immunize a series of 5-day-old rats, followed by subcutaneous inoculation of virulent Hib. The dose of total PRP Ab (Farr technique) required to prevent bacteremia and/or meningitis in 50% of rats (PD50) ranged from 13-916 ng/rat. Sera were also tested for in vitro opsonic activity. Neither protection nor opsonic activity correlated with total PRP Ab. Analysis of anti-PRP IgG, IgG subclass and IgM Ab suggested that high IgG and IgM Ab were associated with protection. A drop in protection was associated with low Ab of two or more IgG subclasses. No single subclass response was consistently protective. High levels of IgG4 and IgM Ab were present in the most opsonic and protective sera. Anti-PRP IgM alone did not compensate for low levels of two or more IgG subclass Ab's. Full protection probably cannot be realized when there are low levels of Ab of 2 or more of the heavy chain genotypes tested (IgM, IgG1-4).

●867 ANTIBODY (AB) RESPONSES OF IGG2 DEFICIENT (G2D) CHILDREN TO H. INFLUENZAE TYPE B (HIB) AND PNEUMOCOCCAL (PN) TYPES 3 (P-3) AND 23 (P-23) POLYSACCHARIDES (PS). Penelope G. Shackelford and Dan M. Granoff, Wash. Univ. Sch. of Med., & Children's Hosp., St. Louis.

In order to investigate the relationship between low serum IgG2 and anti-PS AB deficiency, we compared the IgG1 and IgG2 AB responses of 16 G2D children (mean age=55 mo) vaccinated with Hib PS vaccine with the responses of 51 healthy vaccinated controls (CON) (mean age=35 mo). Total AB responses to Hib PS (measured by Farr assay) were significantly lower in the G2D patients (P<.001, Table). The G2D subjects had significantly lower IgG1 responses (measured by ELISA) than CON (P=.003) and, as expected, the G2D patients also had lower IgG2 responses (P<.001). 11/16 G2D patients (mean age=56 mo) have been vaccinated with PN PS vaccine. The IgG responses to P-3 and P-23 were lower than those of 17 vaccinated CON children (mean age=49 mo),(P<.03 and .09, respectively). Thus, G2D patients have deficient IgG antibody responses to Hib, and P-3 and P-23 PN PS. However, this deficiency is not necessarily related directly to low serum concentrations of IgG2 since G2D patients have deficient IgG1 responses to Hib PS despite normal serum IgG1.

	Hib AB, Geo Mean, µg/ml			IgG PN AB, Geo Mean, 1/Titer	
Subjects	Total	IgG1	IgG2	P-3	P-23
CON	9.1	2.2	0.5	3936	373
G2D	1.5	0.5	0.1	1146	132

868 SEVERE ACQUIRED IMMUNODEFIENCY FOLLOWING LOCAL HIGH DOSE HYPERFRACTIONATED RADIATION THERAPY (HFRT) FOR MALIGNANT BRAIN STEM GLIOMA (MBSG) Kevin Shannon, John Edwards, William Wara, Morton Cowan, Diane Wara. Univ.of Calif.San Fran., Depts of Pediatrics & Radn Therapy. 7200 rads of local HFRT improved projected 2 year disease free survival to 50% in MBSG. Lymphopenia & opportunistic infections in 2 children prompted a study of immune function. Total T cells (TTC) & cytotoxic (CYT), suppressor (SUP), helper (HLP) and inducer (IND) subsets were determined. Proliferative responses were assessed in the mixed lymphocyte reaction (MLR) and with phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Patients were divided into "early"&"late" groups if studies were performed within 6 weeks of RT or later.

	LYMPHS /MM3	%CD3$^+$ TTC	%CD8$^+$ SUP	CYT	HLP	IND	%CD4$^+$ RATIO	4/8	PHA	PWM	MLC
EARLY	(5) 890	50	0	31	14	2	0.53	4527	2103	657	
LATE	(4)1500	50	0	15	25	7	2.13	10874	13347	5597	
NORML	(>)1200	50	2	15	25	13	1.1	48263	10688	6697	

During the period of decreased CD4$^+$ T cells and abnormal mitogen responses, infections included 3 severe interstitial pneumonitis(2 Pneumocystis carinii), disseminated CMV (1), and herpetic pharyngitis (1). Remarkably, no patient had SUP cells as late as 18 mos post RT. Two patients not given decadron during RT had similar, but less severe, immune abnormalities. Local high dose HFRT in MBSG is associated with abnormal cellular immunity & opportunistic infections.

869 SPURIOUS LYMPHOCYTE PHENOTYPES BY FLOW CYTOMETRY FROM MONONUCLEAR CELLS PREPARED BY FICOLL-HYPAQUE. Herbert B. Slade, Jay H. Greenwood, Jerry L. Hudson, Robert H. Beekman III, J. Philip McCoy Jr., Stanley A. Schwartz, University of Michigan Medical School, C. S. Mott Children's Hospital, Departments of Pediatrics and Pathology, Ann Arbor.

Blood mononuclear cells (MNC) are commonly isolated by Ficoll-Hypaque density centrifugation (FH) prior to monoclonal antibody staining and flow cytometric lymphocyte phenotype analysis. We have found that FH separation of peripheral blood is unsuitable for infants less than 4 months of age due to contamination of MNC with red blood cells (RBC's). Identically FH-prepared infant and adult samples showed RBC:lymphocyte ratios of 1.2:1 and 0.2:1 in the MNC suspension on Wright's stained cytospin preparations (n=5). Unlike adult samples, infants' RBC's could not be distinguished from lymphocytes on the basis of forward angle and 90^o light scatter. Each of 8 infant samples was split into paired aliquots prepared by FH and a whole blood technique (WB). The %T3+ was 43.5 +/-17 for FH, 76.8 +/-9 for WB, p=.0047 two sided paired t-test. Results were similar for %T11+, %T4+, %T8+, and %B1+ (Coulter) staining. Differences are attributed to contamination in the FH-prepared lymphocyte gate with non-staining RBC's. No difference was seen in 30 adult samples. RBC lysis should be performed on all blood samples from infants less than 4 months of age.

●870 ONTOGENY OF HUMAN CD4 LYMPHOCYTE SUBPOPULATIONS FOLLOWING BONE MARROW TRANSPLANTATION. John W. Sleasman, Douglas J. Barrett, University of Florida College of Medicine, Department of Pediatrics, Gainesville, Florida.

Human CD4 inducer T lymphocytes consist of two functionally distinct subpopulations. The monoclonal antibody anti-2H4 identifies CD4 cells that induce T suppressor cell function and proliferate in the AMLR, while the monoclonal antibody anti-4B4 identifies CD4 cells that primarily induce B cell help. To define the ontogeny of these inducer subpopulations we studied their number and functional capabilities in seven patients following allogeneic and autologous bone marrow transplantation (BMTx). During the first 90 days following BMTx, both the helper/inducer CD4+ 4B4+ and suppressor/inducer CD4+ 2H4+ subpopulations were elevated (BMTx CD4+ 4B4+ = 75+3% versus control = 42+3%; BMTx CD4+ 2H4+ = 77+5% versus control = 43+2%). However, there was a paradoxical deficiency in the function of the two subpopulations. BMTx patients had a depressed AMLR stimulation index of 3.2+0.5% of control. The post-BMTx CD4+ 4B4+ cells provided poor help for IgG synthesis in a pokeweed mitogen driven co-culture of autologous T and B cells (116+50% of expected IgG synthesis in the BMTx patients versus 465+185% in the controls). Control T cells provided normal help to BMTx B cells (472+176%) but post-BMTx T cells could not provide help for control B cells (135+43%). These data suggest that there is a progenitor cell subpopulation within the CD4 population in post-BMTx that carries both the 2H4 and 4B4 antigenic markers, but does not have the functional capabilities of the terminally differentiated mature CD4 subpopulations.

●871 CONCANAVALIN A ACTIVATES THE SUPPRESSOR/INDUCER SUBPOPULATION OF CD4+ T LYMPHOCYTES. John W. Sleasman, Michelle Henderson, Douglas J. Barrett, University of Florida College of Medicine, Department of Pediatrics, Gainesville, Florida.

Human CD4+ inducer T lymphocytes consist of heterogeneous populations that can be defined either by the monoclonal antibody anti-2H4, which identifies CD4+ cells capable of inducing suppressor T cell function, or by anti-4B4, which identifies CD4+ cells that induce T cell help for B cells. To determine if these subpopulations are terminally differentiated cells within the CD4 lineage, or if they can be modulated by various stimuli, we examined the number and function of the CD4+2H4+ and CD4+4B4+ subpopulations before and after mitogenic activation. Stimulation of CD4+ cells with 10 mcg/ml Concanavalin A (Con A) selectively increased the percentage of cells expressing the CD4+2H4+ phenotype from 43+2% to 82+4%. No change occurred in CD4+4B4+ cells (41+2% to 40+14%). Mitomycin C treatment prior to Con A stimulation abrogated the increase in CD4+2H4+ expression. In contrast to Con A, PHA stimulation produced a non-selective increase in both 2H4 (96%) and 4B4 (92%) expression. Functional studies using autologous T plus B cells in pokeweed driven cocultures, revealed that the Con A activated CD4+ cells induced a T-cell mediated suppression (80+4%) of IgG synthesis. Thus, Con A selectively expands the 2H4+ subpopulation within the CD4+ subset of T cells. The CD4+2H4+ subpopulation may represent a functionally distinct, terminally differentiated suppressor/inducer cell.