836 SYNERGISTIC EFFECT OF STEROIDS AND INTRAVENOUS IMMUNE GLOBULIN (IVIG) IN A PATIENT WITH AUTOIMMUNE NEUTROPENIA (AIN). M. Kallick, R.U. Sorensen and M. Berger, Case Western Reserve University, Department of Pediatrics, Cleveland, Ohio.

IVIG has been used to treat AIN, but it is not always effective. We report a patient in which the concommitant use of IVIG and steroid was more effective than either alone. A 10 year old girl with neutropenia and anti-neutrophil antibodies had chronic gingivitis, buccal erosions, and staphyloccal skin abscesses. Absolute neutrophil counts (ANC) were 40-400/mm^3. IVIG at 2 gm/kg had no effect. She later developed fever, anorexia, and abdominal pain due to bacterial ileitis. Antibiotics alone gave only partial clinical improvement. The addition of Solumedrol, 1 mg/kg/day, transiently raised the ANC from 100 to 3500. The ANC subsequently declined to <100, with clinical relapse, while on the same steroid dose. Adding 1 gm/kg/day IVIG X 3 raised the ANC to 2400 in 2 days and to 11000 in 6 days. This allowed resection of the infected ileum without complications. The ANC remained >1000 for 30 days. Subsequently, while on 40/20 mg alternate day prednisone, 4 infusions of 1 gm/kg IVIG over 7 days did not raise the ANC. Increasing the steroids to 45 mg/day (1 mg/kg) promptly increased the ANC from 800 to 9700 without further IVIG. Ten monthly 1 gm/kg infusions of IVIG have kept her infection-free by maintaining ANC >500, although anti-neutrophil antibodies continue to be present. As prednisone was tapered and discontinued, the ANC response to each course of IVIG decreased. Optimization of prednisone dose in relation to the IVIG infusions is in progress. Our observations suggest that IVIG and steroids may act synergistically to decrease peripheral destruction of antibody sensitized blood cells.

●837 CLINICAL AND IMMUNOLOGIC FEATURES OF PEDIATRIC AIDS-RELATED COMPLEX (PARC). Naynesh Kamani[1], Hylton Lightman[1], Ira Leiderman[2], Leonard Krilov[1]. SUNY at Stony Brook, Schneider Children's Hospital of LIJMC, Dept. of Peds, New Hyde Pk, N.Y.[1], and Mt. Sinai School of Medicine, Division of Clinical Immunology, New York, N.Y.[2] (Spon. by Philip Lipsitz).

The long-term clinical outcome for infants and children with PARC is unknown. We report our detailed clinical and immunologic analyses of 14 patients with PARC (ages 15 mos-8 yrs) who have been followed for 13-66 mos since the onset of their symptoms. Diagnostic criteria for PARC included: 1) clinical features suggestive of Human Immunodeficiency Virus (HIV) infection; 2) presence of serum antibody to HIV; 3) absence of opportunistic infections or malignancies. The most frequent clinical features at presentation were generalized lymphadenopathy (14/14) (GL), hepatosplenomegaly (10/14) (HSM), and recurrent otitis media (7/14). Except for hypergammaglobulinemia (13/14) and reversed T4/T8 ratios (9/14), immunologic analyses, including responses to mitogens and antigens, revealed no consistent abnormalities; 2 pts. had IgG_2 deficiency. Over the course of follow-up, none of the pts. have developed serious or opportunistic infections and all have maintained their growth parameters. The T4/T8 ratios have remained stable (11/13) or improved (2/13). Gradual regression of GL and HSM has been noted in 6 pts. Although large scale follow-up studies over a longer period of time are needed to confirm our observations to date, PARC may represent an end-point response to HIV infection in many infected children. Detailed immunologic evaluation of these pts. may help identify a subset of children that may benefit from therapy such as IV gammaglobulin.

●838 WISKOTT-ALDRICH SYNDROME (WAS) CARRIER DETECTION BY X-CHROMOSOME INACTIVATION ANALYSIS. Donald B.Kohn, Eric R.Fearon, Jerry A.Winklestein, Bert Vogelstein and R. Michael Blaese. NCI, NIH, Bethesda MD, and The Johns Hopkins Hospital, Baltimore, MD.

WAS is an X-linked disorder characterized by immune deficiency, thrombocytopenia and eczema. Previous studies of G-6-PD isozyme expression in rare females doubly heterozygous for WAS and G-6-PD found one of two X chromosomes to be preferentially active in T cells and platelets compared to their fibroblasts. Presumably this is due to selection against cells in which the X chromosome with the WAS allele is active. We have used a new strategy that distinguishes the active and inactive X chromosomes for the detection of WAS carriers. Genomic DNA is analyzed by Southern blot to detect heterozygosity for X chromosome restriction fragment length polymorphisms (RFLP) at the PGK or HGPRT loci. For both loci, the state of methylation is different and constant between the active and inactive X chromosomes. In females heterozygous for these RFLP's the active and inactive X's can thus be distinguished with with methylation-sensitive restriction endonucleases. We have screened 32 female relatives of WAS patients; 14 were heterozygous at one of the two loci (44% potentially informative). The methylation pattern of DNA from T cells of non-carriers showed random X-inactivation. In contrast, DNA from T cells of WAS carriers showed only one of the two X chromosomes to be active. Thus, X-inactivation analysis appears to directly identify WAS carriers and does not require the rare co-occurence of G-6-PD heterozygosity. We are currently using this method to determine the cell lineages affected by the WAS gene defect.

† 839 ESTABLISHMENT AND CHARACTERIZATION OF ADENOSINE DEAMINASE (ADA)-DEFICIENT T CELL LINES. Donald B. Kohn, Jane E. Selegue, Mark Ballow, and R. Michael Blaese. NCI and NHLBI, NIH, Bethesda, Md.

Congenital deficiency of the purine metabolic enzyme ADA causes severe combined immunodeficiency (SCID). The profound T lymphopenia characteristic of this disease has limited direct investigation of the cell most affected by deficiency of ADA. We have been successful in establishing both IL-2-dependent, non-transformed and HTLV-1 transformed T cell lines from eight consecutive ADA-deficient SCID patients. The lines obtained by either method are mature T cells by surface phenotype. These lines all display hypersensitivity to deoxyadenosine, characteristic of ADA deficiency, which is not reversed by deoxycytidine. Clones obtained by limiting dilution of one line show multiple unique rearrangements of the T cell receptor beta gene suggesting clonal diversity among the circulating T cells of this patient, despite lymphopenia and SCID. Lines from 5 patients, all under three years old, have less than 1% normal ADA activity. Pharmacologic inhibitor studies suggest that this remaining activity is attributable to a non-specific aminohydrolase. In contrast, cells from two children ages 11 and 13 years are partially deficient with up to 10% normal ADA activity. An eighth SCID patient, previously found to totally lack erythrocyte ADA has approximately 50% normal ADA in a line derived from thymocytes. Analysis of the molecular basis for ADA deficiency in these lines shows that the majority have a grossly intact ADA gene, contain normal size ADA mRNA (1.6kb) and produce an ADA protein that is catalytically defective.

840 MOLECULAR DETERMINANTS OF IMMUNOGENICITY OF T-INDEPENDENT ANTIGENS IN YOUNG MICE. Howard M. Lederman, Steven K. Bergstrom, Renee Z. Dintzis, Howard M. Dintzis (Spon. by Jerry A. Winkelstein). The Johns Hopkins University School of Medicine, Depts. of Pediatrics, Mol. Biol. and Genetics, and Biophysics. Baltimore, MD

The basis for the impaired antibody (Ab) response of young children to polysaccharide antigens (Ag) is unexplained. A series of dinitrophenyl (DNP)-substituted polyacrylamide (PA) molecules was synthesized to allow comparison of molecular requirements for immunogenicity of polysaccharide-like (T-independent) Ags in young vs adult mice (1-12 wks old). Three DNP-PA Ags were studied which varied in mol. wt. (60, 430, 2000 kD) but had similar hapten density (1 DNP/50 PA monomers). Dose-response relationships and kinetics of both IgM and IgG anti-DNP Ab responses were measured. We conclude that: (a) 60 kD DNP-PA was not immunogenic at any dose in any mouse. (b) Ab responses to 430 kD DNP-PA increased with age up to 10-12 wks when adult maximum levels were achieved. (c) 2000 kD DNP-PA was more immunogenic in every age group than 430 kD DNP-PA. In fact, the response to the 2000 kD polymer in 5 wk old mice was equivalent to the maximum adult response to the 430 kD polymer. (d) 60 kD DNP-PA inhibited the response to optimally immunogenic doses of 430 kD Ags. Inhibition was greatest in youngest mice.

These studies suggest that one strategy to improve immunogenicity of polysaccharide vaccines in young children might involve selective depletion of smaller mol. wt. material (< 100 kD) and/or selective enrichment of large mol. wt. material (> 1000kD) (Supported by grants from NIH and American Lung Association.)

●841 INTERLEUKIN 2 (IL-2) RESTORATION OF DEFECTIVE IN VITRO LYMPHOCYTE PROLIFERATION FOLLOWING BONE MARROW TRANSPLANTATION (BMT). Carl Lenarsky, Kenneth Weinberg, Juanita Petersen, Robertson Parkman. Childrens Hospital of Los Angeles, Division of Research Immunology, Los Angeles, California.

Recipients of allogeneic BMT demonstrate a clinically significant deficiency of cellular immunity after BMT. One in vitro abnormality is defective T lymphocyte blastogenesis to stimulation with mitogens and antigens. Two explanations for this defect are either an absence of cells capable of responding to these stimuli or an absence of cells capable of producing IL-2, the major lymphokine responsible for the clonal expansion of T lymphocytes. We studied the proliferative responses of peripheral blood mononuclear cells from recipients of BMT to both mitogens and antigens. Cells were incubated in the presence and absence of highly purified recombinant IL-2 from E. coli. The addition of IL-2 at concentrations ranging from 10 U/ml to 100 U/ml resulted in an improved blastogenic response to mitogens whenever a less than optimal response was obtained without IL-2. The results with antigens varied according to the elapsed time since BMT. Cells from patients less than one month from BMT did not respond to antigen either in the absence or presence of exogenous IL-2. However, IL-2 at concentrations ranging from 1 U/ml to 10 U/ml did restore the T lymphocyte proliferative response to antigens in patients who were greater than one month post BMT. We conclude that in the first month post BMT lymphocytes capable of responding to mitogens are present, but that IL-2 producing cells are decreased. Lymphocytes capable of responding to antigen first appear one month post BMT, but defective IL-2 production can persist for many months.