848 LOSS OF HELPER CELL FUNCTION IN APLASTIC ANEMIA. Rajendra N. Pahwa and Robert A. Good. Memorial Sloan-Kettering Cancer Center, Immunobiology Division, New York.

Ficoll-Hypaque separated marrow cells from 16 patients and normal volunteers were cultured in soft agar for CFU-c (colony forming units). Marrow cells were further fractionated by velocity sedimentation at unit gravity. Fast sedimenting marrow fractions were cocultured with slowly sedimenting autologous and allogeneic lymphoid cell fractions for 24 hrs, and then plated in soft agar culture. Unfractionated marrow cells from AA patients failed to grow in soft agar. Findings suggestive of a loss of helper cell function were observed in 3 patients; results of one such patient are presented here. The myeloid fraction (sedimentation velocity 5-6.4 mm/hr) of the patient's marrow grew 6.5 colonies/10^5 cells. Coculture of this fast sedimenting fraction with slowly sedimenting normal allogeneic marrow cells (sedimentation velocity < 3mm/hr, morphologically small lymphocytes, no growth in soft agar) resulted in 42.5 colonies/10^5 cells, (i.e. 653% enhancement). In contrast, slowly sedimenting lymphoid cells from the patient's marrow failed to enhance colony formation of autologous myeloid marrow fractions. Thus, in addition to the previously described abnormalities in AA, helper cell defects may also exist in this disorder. Recovery of some patients following treatment with anti-thymocyte globulin and hemi-allogeneic marrow transplantation may be mediated by interaction of helper cells in the transplanted marrow with patient's own stem cells.

● **849** EFFECTS OF SERUM IRON AND TRANSFERRIN ON BACTERIOSTASIS: IN VIVO-IN VITRO DIFFERENCES Howard A. Pearson, Robert Baltimore, Duane G. Shedd Yale University School of Medicine, Department of Pediatrics, New Haven, CT

Human serum inhibits the growth of a variety of microorganisms, including many pathogens. To a large extent, bacteriostasis is due to serum transferrin (Tr), which binds iron tightly, making it unavailable to bacteria which require the metal for nucleoprotein synthesis and growth. Addition of exogenous iron to serum abolishes its bacteriostasis. Weinberg's hypothesis of "nutritional immunity" postulates that unsaturated Tr is protective against infection and cautions that iron treatment might reduce host resistance by increasing serum iron. In most reported studies, iron has been added to serum *in vitro* rather than employing sera with varying intrinsic iron contents. We confirmed that normal heat-treated human serum inhibited growth of two pathogenic *E. coli* isolates. Addition of iron to saturate Tr abolished the bacteriostatic properties. In contrast, we found no differences in the rates of growth of a standardized inoculum of *E. coli* in sera from 3 iron-deficient, 17 normal, and 13 iron-overloaded individuals (serum Fe 26-349 µg/dl, Tr saturation 6-100%). Iron added to serum *in vitro* is probably non-specifically bound to several proteins from which bacteria can easily extract it. However, *in vivo*, the metal is completely and firmly complexed to Tr and hence non-available. The contention that ordinary iron treatment predisposes children to infection is not supported by a body of epidemiological evidence. Our studies provide experimental data which cast further doubt on "nutritional immunity" as an important host defense mechanism.

850 THYMOCYTE ANTIGEN IN T CELL LEUKEMIA. Joanne K. Pincus, John M. Falletta, Richard Metzgar, Jeanette Pullen, William Crist, G. Bennett Humphrey, Jim Boyette, and Jan van Eys, Duke University Medical Center, Durham and the Pediatric Oncology Group, St. Louis.

Two hundred seventy-seven pediatric patients with newly diagnosed acute lymphocytic leukemia were examined for the presence of T lymphocyte associated antigen (TLAA) and thymus antigen (Thy) on their bone marrow leukemic blasts. These antigens were identifed with carefully absorbed xenoantisera and monoclonal antibodies directed against human peripheral T cells and thymocytes, respectively. 227 patients were TLAA (-) and Thy (-). 50 patients had T cell leukemia (positive for TLAA). Of these, 21/50 (42%) were Thy (+); 29/50 (58%) were Thy (-). These two T cell subgroups were compared regarding previously recognized features of T cell disease:

% of Patients:

	%ER <20	%ER >40	CALL (+)	Med. Mass+	WBC >100,000	Age <2, >10 yrs	Sex M	Relapse	Disease Deaths
Thy (+)	10	76	5	62	60	28	85	25	10
Thy (-)	50	27	28	11	29	30	68	49	30

Thy antigen positivity redefines "typical" T cell disease. Thy Ag negativity selects a previously undefined subgroup of patients with T cell disease, theoretically describing a more differentiated blast cell line, with the suggestion of an even poorer response to conventional therapy in the small group of patients examined. Ongoing studies of patients with TLAA (+) Thy (-) disease will more completely define this subgroup.

● **851** EXERCISE-INDUCED HEMOLYSIS IN SICKLE CELL DISEASE. Orah S. Platt, Samuel E. Lux, and David G. Nathan. Division of Hematology and Oncology, Harvard Medical School, Children's Hospital, Boston, MA.

Investigation of a patient with sickle cell disease (SS) and hemoglobinuria led us to realize that SS patients may develop exercise-induced hemoglobinemia. 6 patients: 4 SS and 2 Hgb SC (SC) were studied before and after exercise. All had plasma Hgb <5 mg% initially. After exercise plasma Hgb in SS patients rose 10-16 mg% but remained <5 mg% in SC. To see if patient's RBCs were abnormally fragile when exposed to shear forces that could be generated in the small vessels of exercising muscles, we exposed them to physiologic shear rates in a coneplate viscometer (Hct 70, 37°C, pO_2 100) for 2 min. Normal and SC RBCs had no hemolysis at shear stresses less than 2000 dynes/cm2 (d/cm^2) and 1% at 2300 d/cm^2. Hemolysis of SS RBCs began at 700 d/cm^2 and continued linearly to 7% at 2300 d/cm^2. This shear/hemolysis profile is comparable to that of xerocytes and artificially dehydrated RBCs. SS RBCs separated on Stractan gradients had populations of dehydrated cells that were increasingly shear fragile with increasing dehydration. The abnormal shear/hemolysis profile of these dehydrated layers was normalized by in vitro rehydration using nystatin and a high K buffer. Such dense and fragile layers were not found in SC or normal blood. These data suggest that the exercise-induced hemolysis in SS patients is related to lysis of dehydrated, shear-sensitive cells. This same process may also contribute to the chronic hemolysis of SS - a phenomenon known to correlate with numbers of dehydrated, irreversibly sickled cells.

852 THROMBOCYTOSIS IN PATIENTS WITH SICKLE CELL ANEMIA AND PNEUMONIA. Mortimer Poncz, Jay Greenberg, Marie O. Russell, Elias Schwartz, and Alan R. Cohen. University of Pennsylvania School of Medicine and The Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia.

Alterations in platelet function in patients with sickle cell anemia (SCA) may contribute to vaso-occlusive crises. Since thrombocytosis may further enhance thrombotic tendencies, we investigated the occurrence rate, magnitude and adverse effects of thrombocytosis in children with SCA and pneumonia. In 37 patients, 47 episodes of pneumonia were evaluated. Platelet counts in 7 patients rose to greater than 1 x 10^6/mm^3 and in 16 patients to ≥750,000/mm^3. The mean platelet count began to rise on the fifth day. From day 7 to day 21 the average platelet count was greater than twice the average count on admission and during asymptomatic periods. There were no platelet aggregation abnormalities detected initially or after the development of thrombocytosis. The mean platelet volumes, determined on an electronic particle counter, also remained constant. Only one patient had a painful crisis during the duration of post-pneumonia thrombocytosis. His painful crisis resolved despite a continued rise in platelet count. Although thrombocytosis following pneumonia in patients with SCA is common, the risk of related vaso-occlusive sequelae appears low and anti-platelet therapy does not appear warranted.

853 PNEUMOCOCCAL SEPTICEMIA IN CHILDREN WITH SICKLE CELL ANEMIA. CHANGING TREND OF SURVIVAL. Darleen Powars, Gary Overturf, Jose Rigau-Perez, Linda Chan, University of Southern California School of Medicine, Los Angeles, Ca.

Streptococcus pneumoniae infection has been the predominant cause of death among children with sickle cell anemia (SS). We report our observed change in the pattern of progression of septicemia to meningitis and death in non-immunized SS children who were not on prophylactic penicillin in the face of a persistently high incidence of pneumococcal disease. Of 233 SS children less than age 6 observed for 781 person years, the overall incidence rate of pneumococcal septicemia was 5.9 episodes per 100 person years. Prior to July 1972, of 23 children who had pneumococcal septicemia, 8 (35%) died and 15 (65%) developed meningitis, whereas since July 1972, 11 children have had pneumococcal septicemia, but no children died and only 2 (18%) developed meningitis ($P<0.05$). This decrease in major morbidity is attributed to the establishment of a clinical program which provides close medical supervision of the SS child with fever and the rapid institution of parenteral antibiotic therapy.