● 734 HEMOPHILIA A: RECURRENT MUTATIONS AND AN UNUSUAL DELETION. H. Youssoufian, A. Patel, D. Phillips, H. H. Kazazian, S. E. Antonarakis. Johns Hopkins Univ. Sch. of Med., Dept. Pediatrics, Baltimore.

We have identified 14 mutations of the factor VIII (F8) gene from a panel of 107 patients with hemophilia A and have characterized these gene defects by restriction analysis, oligonucleotide hybridization, cloning and DNA sequencing. Recurrent point mutations that involve CG to TG transitions were identified in exon 18, exon 22 and exon 24, and a single CG to TG transition was identified in exon 23. In addition, a Taq I site alteration in intron 4 of the F8 gene was identified in a patient with mild hemophilia. Cloning and sequencing of this region suggests that a change in this Taq I site may create a new splice donor site. These data suggest that CG to TG transition is a prominent mechanism of mutation in hemophilia A. We estimate that the mutation rate of CG to TG in the F8 gene is at least 100 times greater than the average mutation rate per nucleotide. Six different deletions were also characterized. In one family, the deletion involved exon 26. However, the deletion endpoints in the male proband were different from those in his carrier mother, suggesting either gonadal mosaicism or a second deletion event in maternal meiosis. Of the 14 mutations, 6 occurred de novo within 2 generations; 4 in males and 2 in females. In these de novo mutations paternal age at conception was 35 (range = 32-38) and maternal age was 24 and 27. The ability to discover a sizable number of mutations in the F8 gene producing hemophilia A enables us to determine the frequency and nature of de novo mutations in man.

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735 RED CELL SIZE HETEROGENEITY DURING ONTOGENY. Blanche P. Alter, James D. Goldberg and Richard L. Berkowitz, Mount Sinai School of Medicine, Departments of Medicine, Pediatrics, and Obstetrics, Gynecology, and Reproductive Science, New York.

To determine the manner in which erythrocyte changes occur during ontogeny, several red cell parameters were analyzed in 19 fetuses, 14 newborn infants, 19 children and 45 adults. Although MCV and Hb F levels decreased as expected during in utero development, the coefficient of variation of red cell size (%CV = SD/mean), or red cell distribution width (RDW), increased from fetuses to newborn infants, and then decreased in children to adult levels. In normal children and adults, in which erythropoiesis was in steady-state, the %CV was 15 (RDW was 13). The %CV in fetuses at 18 to 24 weeks gestation was 18, and it was 21 at term birth. This high value for %CV in newborn infants indicates substantial anisocytosis. Erythropoiesis at the time of birth is not a steady-state condition. Erythrocytes of a wide variety of sizes are present, with the appearance of new small cells on a background of older, larger red cells. This increased anisocytosis suggests that these new erythrocytes do not appear due to a smooth, continuous evolution of red cell size, but due to discrete, perhaps clonal changes.

736 ERYTHROCYTE CHARACTERISTICS IN CHILDHOOD ACUTE LEUKEMIA. Blanche P. Alter, Michael A. Weiner and Michael B. Harris, Mount Sinai School of Medicine, Departments of Medicine and Pediatrics, New York.

Children with acute leukemia often have erythrocytes with 'fetal-like' features. To examine the relationship of the type and phase of the leukemia, we studied 39 children with newly diagnosed acute lymphocytic (ALL) and 5 with acute nonlymphocytic leukemia (ANLL). 25 patients were evaluated during chemotherapy, 3 off therapy, and 12 at relapse. Macrocytosis and/or anisocytosis (increased coefficient of variation of red cell size, or %CV, ie SD/mean) were seen in 70% of new ALL, 80% of new ANLL, and 90% of ALL and 100% of ANLL on treatment. F-cells (acid elution) were increased in 24% of ALL and 80% of ANLL at diagnosis, and 61% of ALL and 75% of ANLL during chemotherapy. Hb F levels (alkali denaturation) were elevated in 8, 40, 29 and 33% respectively. Nonleukemic controls for chemotherapy (6 patients with osteogenic sarcoma) all had macrocytosis and/or anisocytosis, and 83% had increased F-cell proportions, although Hb F was normal in all. Features at leukemic relapse on chemotherapy were similar to those during treated remissions. Abnormal red cells are common at diagnosis in ANLL, where they may belong to the malignant clone, and ALL, where stress erythropoiesis and/or leukemic factors may be contributory. During chemotherapy, drug-related erythrocyte changes and marrow suppression and recovery are added to factors due to leukemia itself. Thus leukemia, chemotherapy, and the combination lead to macrocytosis, anisocytosis, and 'fetal-like' erythrocytes.

737 MEASUREMENT OF L-ASPARAGINE (ASN) LEVELS IN THE PRESENCE OF E. COLI L-ASPARAGINASE (ASNASE) IS IMPROVED BY THE USE OF ASPARTIC β SEMIALDEHYDE (ASA). Barbara L. Asselin, John C. Whitin, and Harvey J. Cohen. University of Rochester Medical Center, Strong Memorial Hospital, Department of Pediatrics, Rochester, NY

The anti-leukemic activity of ASNase is thought to result from depletion of serum ASN. In studies of the pharmacologic effects of ASNase, investigators have reported dramatic prolonged reduction in the serum concentration of ASN after the administration of ASNase. Such measurements, however, have not accounted for the problem of hydrolysis of ASN by ASNase that is present in blood samples. We examined recovery of ^{14}C ASN from blood samples with and without added ASNase at various concentrations. In the presence of >0.001 IU/ml of ASNase, the amount of ^{14}C ASN recovered was <20% of that without ASNase, within 5 minutes. Utilizing this assay we have studied the effect of several known inhibitors of ASNase in an attempt to improve ASN recovery. In the presence of ASA, ASN levels in the presence of 0.001 to 1.0 IU/ml of ASNase remain at >90% of control. Blood samples drawn from patients at 1 and 7 days following ASNase injection were collected directly into tubes containing ^{14}C ASN +/- ASA. Recovery with ASA, was 60-110% and 100-110% of control (i.e., no ASNase) on days 1 and 7 respectively. We conclude that: 1) continued hydrolysis of ASN by ASNase can result in falsely low serum ASN measurements; 2) ASA is a potent inhibitor of E. Coli ASNase; 3) ASA present in collection tubes obviates the problem of continued ASNase activity. Thus, for accurate measurements of the rate and degree of ASN depletion by ASNase, ASA should be present in the blood collection system.

● 738 EFFECT OF MAINTENANCE CHEMOTHERAPY(C)FOR ACUTE LYMPHOBLASTIC LEUKEMIA(ALL)ON INTERLEUKIN-2(IL-2)PRODUCTION, RESPONSE AND RECEPTOR EXPRESSION. Michael S. Borzy, D.L. Ridgway, C.A. Parkman-Newton, M.T. Gregg(Spon. by R.C. Neerhout), Oregon Health Sciences University, Department of Pediatrics, Portland.

This study was undertaken to define both the acute and long-term effects of C on the IL-2 system. 10 children with ALL receiving standard C for 6-36 mos, 11 children with All off all C for 2-80 mos and 15 age-matched controls were studied. Responsiveness to IL-2 was determined by culturing activated T cells with human recombinant IL-2 with proliferation estimated by ^{3}H-TdR incorporation. IL-2 production was quantitated in culture supernatants from phytohemagglutinin(PHA)-stimulated mononuclear cells(MNC). IL-2 receptor(IL-2R)expression was quantitated on PHA-stimulated MNC.

Cells	IL-2 Response(CPM)	IL-2 Production(U/ml)	IL-2R(%)
ALL on C (N=10)	9,876[a](2,399-40,663)[b] (p=0.05)[c]	5.5 ± 7.6[d] (p=0.02)	18.2± 7.6[d] (p=0.01)
ALL off C (N=11)	33,466 (12,212-91,706) (p=NS)	4.9 ± 6.9 (p=0.02)	19.9±12.8 (p=0.01)
Controls	40,883 (33,251-50,269)	11.1 ± 9.0	30.8± 13.1

a=geom. x̄; b=95% confidence interval; c=vs control; d=x̄ ± S.D.

In conclusion, these results demonstrate that children with ALL receiving C have a significantly depressed IL-2 system. These results also show that although cessation of C allowed IL-2 responsiveness to normalize, the defects in IL-2 production and IL-2R expression persisted, suggesting that a substantial therapy-induced immunodeficiency may be among the late effects present in long-term survivors of ALL.

739 FAVORABLE RESPONSE TO IVIgG IN A WISCOTT-ALDRICH SYNDROME INFANT WITH SEVERE THROMBOCYTOPENIA REFRACTORY TO SPLENECTOMY. Gordon L. Bray, Edythe Albano, R. Michael Blaese. Division of Hematology-Oncology, Child Hosp Natl Med Ctr, Washington, DC, and Metabolism Br, NCI, Bethesda, MD.

Other than bone marrow transplantation, splenectomy is the only effective, long term therapy for the severe thrombocytopenia of Wiscott-Aldrich Syndrome (WAS). Occasional WAS patients demonstrate continued severe thrombocytopenia, either immediately post-splenectomy (p-s) or following an initial improvement in platelet count. These patients may respond to less satisfactory treatment modalities such as steroids or other immunosuppressive agents. We recently evaluated a WAS patient with persistent, severe thrombocytopenia p-s who demonstrated a favorable response to Sandoglobulin^{R} (IVIgG). The diagnosis of WAS was based on the following criteria: eczema noted at one month of age, thrombocytopenia and mucous membrane bleeding beginning at two months of age, tiny platelets on peripheral blood smear and a bone marrow aspirate demonstrating normal megakaryocyte numbers and morphology. Bleeding episodes were treated with platelet transfusions until 9 mos. of age at which time splenectomy was performed. Severe thrombocytopenia (platelet count (PC) < 20,000/mm3) persisted p-s requiring continued platelet transfusions. IVIgG, 1gm/kg/day for 3 consecutive days, was given 5 days, 4 wks, and 7 wks p-s. Post infusion, peak PCs (238,000, 171,000 and 36,000/mm3 respectively) were noted 1-3 days following each course of IVIgG; no platelet transfusions were required during the treatment period. The potential benefits of this modality justify its further investigation in the p-s, WAS patient with persistent thrombocytopenia.