912 SIMILAR DECREASE OF ANTITHROMBIN III (ATIII) AND ALBUMIN IN BIRTH ASPHYXIA AND PRESUMED DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

Barbara K. Schmidt, Toshihiro Muraji, Alvin Zipursky
The Hospital for Sick Children, Departments of Pediatrics and Surgery, Toronto, Canada

Low ATIII levels in critically ill patients are usually ascribed to consumption during DIC and have been recommended as a diagnostic aid in that disorder. We compared plasma ATIII and albumin levels in 48 newborn rabbits with either no or various degrees of asphyxia and resultant acidosis (pH 6.70-7.30). Both ATIII and albumin levels (albumin 5-21 g/l) were markedly decreased in the sickest animals and there was a direct linear relationship between the two proteins ($p<.001$). The hematocrit (0.37-0.52) was directly and linearly related to albumin, suggesting expansion and dilution of the plasma pool ($p<0.01$). 8 neonates with the clinical and laboratory diagnosis of DIC complicating asphyxia or severe infection were also studied. Both ATIII and albumin were decreased below the reference range in all cases. Rather than intravascular consumption, increased nonspecific protein losses (capillary leakage, decreased entry into the plasma space) and/or dilution appear to be responsible for the observed falls in plasma proteins. Our findings discredit ATIII as a useful diagnostic marker of neonatal DIC. Further, a similar behaviour of clottable and non-clottable proteins in shock and other non-steady states questions the general assumption, that the ensuing coagulopathy is due to DIC.

● 913 INTRAVASCULAR COAGULATION AND COMPLEMENT ACTIVATION IN SYSTEMIC ONSET JUVENILE ARTHRITIS (S-JA).

J. Paul Scott, Carlos Arroyave, Mona Maryjowski, Patricia Gerber, Lauren M. Pachman. Northwestern University Medical School, the Children's Memorial Hospital, Department of Pediatrics, Chicago, IL.

We observed an episode of acute disseminated intravascular coagulation and purpura fulminans in a child with S-JA. We had previously reported the association of thrombocytosis in juvenile arthritis (JA). These findings prompted us to investigate whether there is evidence of intravascular coagulation in association with complement activation in JA. We prospectively studied 10 children with S-JA, 10 children with polyarticular JA (P-JA) and 10 age/sex matched controls. In addition to routine clotting studies, we measured plasma fibrinopeptide A (FPA), a sensitive measure of thrombin generation, factor VIII related antigen (VIIIR:Ag), an endothelial cell protein, and platelet factor 4 (PF-4), a platelet secreted protein. The complement split products, C3d and C4d, were also assayed to indicate complement activation. The following data were obtained:

Group	PT sec	PTT sec	Fibr. mg/dl	VIIIR:Ag u/dl	FPA ng/ml	PF-4 ng/ml	C-split Products
S-JA	14 + 1.5	47 + 11*	415 + 81**	406 + 250**	9.7 + 8**	7.7 + 3	+ 7/7
P-JA	12 + 0.5	40 + 6.5	286 + 55	83 + 25	3.7 + 2*	8.5 + 6	+ 2/4
Control	12 + 0.8	37 + 4	217 + 38	118 + 73	1.9 + .5	6.0 + 3	- 4/4

* $p<.05$ ** $p<.01$ when compared to controls

In the patients with S-JA and P-JA, the mean platelet counts and disease activity were similar in each group. We conclude: 1) activation of coagulation is common in S-JA but not P-JA and may cause severe morbidity; 2) complement is also activated in S-JA; 3) marked elevations of factor VIIIR:Ag suggest disturbance of the vascular endothelium in S-JA but not P-JA; 4) normal PF-4 levels do not suggest intravascular platelet consumption in either P-JA or S-JA.

● 914 FOUR DRUG CHEMOTHERAPY, TOTAL BODY IRRADIATION (TBI) AND ALLOGENEIC OR AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) FOR METASTATIC NEUROBLASTOMA.

R.C. Seeger, C. Lenarsky, T.J. Moss, S.E. Siegel and J. Wells. Department of Pediatrics, UCLA , Los Angeles, CA 90024.

Intensive chemotherapy, TBI, and BMT may improve the outcome for children with metastatic neuroblastoma (August, et al, 1982). We are testing a new four drug chemotherapy and TBI pretransplant regimen for its toxicity and efficacy. Five patients received cis-platinum, VM26, doxorubicin, melphalan, and TBI (VAMP-TBI); and 3 received melphalan and TBI (M-TBI) because they could not tolerate the other agents or because their tumor was judged resistent. Allogeneic (allo) marrow was given to 5 and autologous (auto) marrow to 3 patients. They were 1 1/2 to 7 yrs old when transplanted (median 5 yrs) and were transplanted 5-13 mos after diagnosis (median 10 mos). Auto marrow had no neuroblastoma cells by immunoperoxidase staining for neuron specific enolase and cell surface antigens, which detects 1 tumor cell/10^5 normal cells. The most consistent acute toxicity from VAMP-TBI was severe mucositis, vomiting, and diarrhea; M-TBI also caused these complications but to a lesser extent. One acute toxic death occurred with each regimen. Of the 5 patients receiving VAMP-TBI, 3 have no evidence of disease (NED) at 60 (auto), 195 (allo), and 317 (allo) days; and 1 (auto) is 7 days post transplantation. One of 3 receiving M-TBI is NED at 45 days (auto), and another has progressive disease at 189 days (allo). We conclude that VAMP-TBI is a tolerable conditioning regimen. The survival of 3 of 4 evaluable patients receiving VAMP-TBI with NED suggests that this regimen should continue to be investigated.

● 915 THE SILENT CARRIER ALLELE: β-THALASSEMIA WITHOUT A MUTATION IN THE β-GLOBIN GENE REGION.

Gregg L. Semenza, Kathleen Delgrosso, Saul Surrey, Padmini Malladi, Elias Schwartz, and Mortimer Poncz. University of Pennsylvania School of Medicine and Children's Hospital of Phila.

The silent carrier of β thalassemia has a decreased β/α globin synthesis ratio, but normal Hb A_2 and Hb F levels and red cell indices. We have restudied the first described family (N. Engl. J. Med. 281:1327, 1969), in which the father is a silent carrier, the mother has high Hb A_2 β-thalassemia trait, and both children have β thalassemia of intermediate clinical severity. The relative excess α globin in this family is not due to an increase in α-globin gene numbers. The maternal and paternal β-globin genes were cloned from the daughter's genomic DNA. The maternal gene contains a previously reported IVS-1 splice junction β^o thalassemia mutation. Sequence analysis of the paternal gene failed to reveal any base changes of functional significance. In HeLa cells the gene was expressed at normal levels with proper processing of RNA. Haplotype analysis revealed that the affected son and daughter inherited different εγδβ-globin gene clusters from the father. However, the father was homozygous for all polymorphic restriction sites downstream from a Taq I site approximately 3 kb 5' to the δ-globin gene. Two explanations for these results are: (1) recombination within primordial germ cells of the father occurred downstream from the Taq I site (2) the paternal silent carrier allele is not linked to the β-globin cluster. Studies which distinguish between these possibilities are in progress. In either case, the mutation responsible for β thalassemia is outside the region analyzed by structural and functional studies.

916 ADEQUACY OF CHEMOPROPHYLAXIS ALONE IN PREVENTION OF CENTRAL NERVOUS SYSTEM (CNS) LEUKEMIA IN CHILDREN WITH STANDARD RISK ACUTE LYMPHOCYTIC LEUKEMIA (S-ALL).

Ashok Shende, Robert Festa, Gungor Karayalcin and Philip Lanzkowsky. School of Medicine, Health Sciences Centre, SUNY at Stony Brook, Children's Hospital of LIJ-HMC, Dept of Peds, New Hyde Park, N.Y.

The neurotoxicity resulting from the combination of cranial irradiation and intrathecal methotrexate (IT MTX) for prophylaxis of CNS leukemia led to the use of IT MTX alone in S-ALL (WBC<50,000/mm^3). From July 1978 to February 1983 thirty-nine children with S-ALL (14 boys, 25 girls; ages 1-6/12 to 14-5/12 years; WBC 900-37,200/mm^3) received IT MTX alone for CNS prophylaxis. All the patients received the same induction, consolidation and maintenance multi-drug systemic treatment and IT MTX alone for CNS prophylaxis. Therapy was discontinued at 3 years in those patients remaining in complete continuous remission. With a median observation period of 32+ months the following relapse rate was observed: a) isolated CNS: 3 patients (7.6%) at 11, 22, and 35 months; b) simultaneous CNS and bone marrow (BM): 1 patient (2.5%); c) BM alone: 13 patients (33%). The isolated CNS relapse rate of 7.6% utilizing IT MTX alone in S-ALL compares favorably with studies utilizing a combination of cranial irradiation and IT MTX. We conclude that the administration of IT MTX during induction and maintenance therapy provides adequate CNS prophylaxis for S-ALL and justifies the ommission of cranial irradiation in these children.

917 LONG TERM HUMAN BONE MARROW CULTURE (LTHMC) USING BONE MARROW ASPIRATES FROM CHILDREN.

Takeo Shibata, Susumu Inoue. Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan 48201.

To investigate whether small volumes of marrow aspirates are suitable for LTHMC, we cultured 22 marrow aspirates from children with a variety of hematological disorders according to the method of Coulombel et al (Blood 62:291, 1983). 5-40x10^6 buffy coat cells recovered from 3-4 ml of marrows were cultured in α medium with 10^{-6} Mol hydrocortisone, 12.5% horse serum and 12.5% fetal calf serum in 35mm dishes at 2x10^6 cells/dish. Demidepopulation of nonadherent cells was done weekly. Differentials on nonadherent cell showed early and late myeloid cells till 5th week, after which 95-98% of the cells were macrophages. An increase in the lymphocytes proportion for the first 3-4 weeks was observed. Studies of 3 specimens with monoclonal antibody OKT-3 revealed positive cells in 0 of the 3 nonadherent fractions, and 0.1% positive cells in adherent fraction in 1 at 2-3 week of culture. Adherent cell layer with nests of small round cells in "cobblestone-like" arrangement was established in 18 of 22 specimens within 2 weeks. There was clear association between the presence of the cobblestone-like cells and generation of CFU-C colonies by adherent cells. None of 5 adherent specimens without these round cells formed CFU-C colonies, while 3 of 3 adherent specimens with the cobblestone-like cells formed 5-9 colonies/5x10^4 cells plated. We conclude that establishment of LTHMC is possible with a small volume of aspirated marrows from pediatric patients, and that presence of cobblestone-like cells indicates generation of stem cells in the adherent layer.