89 HEREDITARY RED BLOOD CELL ADENOSINE DEAMINASE OVERPRODUCTION: ASSOCIATION WITH INCREASED LEVELS OF STRUCTURALLY NORMAL ADA mRNA. E.G. Chottiner, D.S. Ginsburg, and B.S. Mitchell, University of Michigan, Department of Internal Medicine, Ann Arbor, Michigan, U.S.A.

A marked tissue-specific increase in erythrocyte adenosine deaminase activity is associated with an autosomal dominantly inherited hemolytic anemia. Preliminary studies suggested that the defect lay at the level of ADA mRNA translation in red blood cells. We have directly analyzed reticulocyte ADA mRNA from affected individuals. Sequencing of seven proband reticulocyte ADA cDNA clones did not reveal any base changes in coding or non-coding regions. RNase mapping demonstrated that the 5'- and 3'-untranslated regions of reticulocyte and B lymphoblast ADA mRNAs from affected individuals were identical to those of normal B lymphoblasts. However, RNase mapping failed to detect any comparable bands in RNA from control reticulocytes. Northern blot analysis performed under stringent hybridization and washing conditions confirmed a markedly increased amount of reticulocyte ADA mRNA in affected individuals compared to controls. These findings were obscured in the previous study by non-specific hybridization of the ADA cDNA probe to 18s ribosomal RNA. We conclude that the red cell-specific overexpression of ADA probably occurs at the level of transcription in erythroid precursors.

90 METABOLISM OF GUANINE ARABINOSIDE AND CYTOSINE ARABINOSIDE IN CELLS FROM PATIENTS WITH LEUKEMIA. Donna S. Shewach and Beverly S. Mitchell, University of Michigan Medical Center, Ann Arbor, Mi. USA.

Guanine arabinoside (araG) and cytosine arabinoside (araC) are nucleoside analogs which elicit cytotoxicity through their corresponding 5'-triphosphate metabolites. Although less potent than araC, araG is of interest as an antileukemic agent due to its selective toxicity to cultured T compared to B lymphoblasts. In order to determine whether araG would exhibit a similar selectivity of action in vivo, we have examined the metabolism of this drug in mononuclear cells from patients with leukemia. Peripheral blood was diluted and incubated with 100 μM araG or 10 μM araC for 4 hours. The highest level of araGTP accumulation occurred in leukocytes from patients with T-cell ALL (median value 187 pmol/10^7 cells). Cells from patients with AML or non-T-, non-B cell ALL accumulated lower levels of araGTP (72 and 86 pmol/10^7 cells, respectively), and cells from patients with CLL accumulated the lowest amount of araGTP (31 pmol/10^7 cells). In contrast, accumulation of araCTP was similar in cells from patients with T-cell ALL, AML and non-T, non-B-cell ALL. The amount of araG or araC remaining in plasma after 4 hours varied and was not related to the cellular accumulation of the corresponding nucleotides. These results suggest that araG may act as a selective chemotherapeutic agent in patients with T-cell ALL.

91 EFFECTS OF IMP DEHYDROGENASE INHIBITION ON THE DIFFERENTIATION OF HL-60 PROMYELOCYTIC LEUKEMIC CELLS. T.E. Gan amd B.S. Mitchell, University of Michigan Medical Center, Ann Arbor, MI.

HL-60 promyelocytic leukemic cells have been demonstrated to differentiate in association with depletion of guanine ribonucleotide pools induced by inhibitors of the enzyme IMP dehydrogenase. We have asked whether GTP depletion is indeed causal of HL-60 cell maturation induced by the IMP dehydrogenase inhibitor mycophenolic acid (MPA) and have compared the effects of MPA on cellular metabolism with those of other differentiating agents. Cells grown in the presence of 2 μM MPA were 85 ± 6% differentiated on day 4 of culture. GTP pools were reduced to <20% of control values by 1 hr and remained low over the 4 day incubation period. Repletion of guanine nucleotide pools within 24 hr of initiation of the cuture by exogenous guanosine in the presence of MPA prevented differentiation in wild type but not in HPRT-deficient HL-60 cells. There was no depletion of GTP associated with other differentiating agents and no reversal of differentiation with guanosine. MPA treatment did not result in significant alterations in intracellular cAMP, but did decrease cGMP to 50% of control levels at 24 hrs. There were no changes in ^{32}P-labelled protein patterns on autoradiographs of two dimensional protein gels after MPA exposure. We conclude that depletion of guanine nucleotides is causally related to the HL-60 differentiation induced by IMP dehydrogenase inhibitors but not by other inducers of differentiation of these cells.

92 REDUCED PURINE NUCLEOTIDE CONTENT AND POLY(ADP-RIBOSE) SYNTHETASE ACTIVITY IN HPRT-DEFICIENT HUMAN LYMPHOBLASTS

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HPRT-deficient EB-virus transformed human lymphoblasts show decreased levels of ATP and GTP compared with control lymphoblasts. This decrease was not consistent.

The ATP content of the HPRT-deficient cells decreased to a significantly lower level than in control cells under unfavourable nutritional conditions, but not with frequent changes of medium. There was also a significant decrease in ADP-ribosylation in these cells.

The cells showed a significant positive correlation between ATP content and poly(ADP-ribose) synthetase activity and an inverse correlation between NAD content and poly(ADP-ribose) synthetase activity, as NAD is the substrate for this enzyme.

ADP-ribosylation is essential in many cellular processes including DNA repair and cell differentiation. So a decrease in ADP-ribosylation of nuclear protein may lead to decreased DNA repair. This may be a factor in explaining the long term deterioration of brain and testicular function in the Lesch-Nyhan Syndrome.

93 MOLECULAR ASPECT OF MYOGENIC HYPERURICEMIA: CLONING OF HUMAN MUSCLE PHOSPHOFRUCTOKINASE cDNA

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Our recent investigations revealed that myogenic hyperuricemia (Mineo et al.) is a common pathologic feature of glycogenosis types III, V and VII, in which muscle glycolysis is impaired. To clarify the molecular defect in glycogenosis type VII (human muscle phosphofructokinase (HMPFK) deficiency), we began performing the molecular cloning of HMPFK cDNA.

A human muscle cDNA library was prepared by Okayama and Berg method and screened by colony hybridization with a rabbit muscle PFK cDNA (a gift from Dr. S. D. Putney of the Repligen Corp. MA, U.S.A.). A partial HMPFK cDNA clone was isolated. The clone was primer-extended and two other overlapping clones to cover the full-length were isolated.

Complete primary structure of the enzyme was determined through the sequence analysis. HMPFK was 85,050 Da in size with 779 amino acid residues. Internal homology between N- and C-halves of the peptide was observed as in rabbit muscle PFK. Sequence homologies between rabbit and human muscle PFK were 96% in amino acids and 89% in nucleotides.

Cloning of HMPFK cDNA will help analyzing the pathological regulatory mechanism of muscle glycolysis responsible for the development of myogenic hyperuricemia at the molecular level.

94 CHANNELING OF IMP INTO GUANYLATE SYNTHESIS IN THE GROWTH PROGRAM OF HEPATOMA 3924A CELLS

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The purpose was to elucidate the relationship of IMP utilization for de novo guanylate and adenylate synthesis during the display of the proliferative program of hepatoma 3924A cells. Purine compounds in the cells were labeled with [^{14}C]formate or L-[3-^{14}C]serine in a serine-free assay medium, hydrolyzed to purine bases, separated and counted. Under these assay conditions labeling of adenine or guanine was proportionate with incubation time and with number of cells. During the transition of hepatoma cells from plateau phase into logarithmic proliferation the concentration of PRPP increased 14-fold, followed by an 8-fold rise in purine de novo synthesis. In plateau phase cells predominantly adenylates were produced. After replating the resting cells there was a sharp increase in the relative labeling of guanine with a concurrent marked decrease in that of adenine in the late lag phase. The subsequent decline of the relative labeling of guanine was accompanied with the restoration in that of adenine with the increase in the cell density. The striking redirection in the distribution of label from IMP utilization to the preferential synthesis of guanylates during the expression of the biochemical proliferative program of cancer cells underlines the potential significance of this pathway as a target of chemotherapy.

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