49 GENE TRANSFER, AMPLIFICATION, AND AN ANALYSIS OF MUTANTS IN THE UMP SYNTHASE GENE.

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To isolate clones of the human UMP synthase gene, we have produced an amplified, human secondary transformant for the human UMP synthase gene using DNA mediated gene transfer and drug resistance to azauridine. The secondary transformant has 35-fold increased levels of ODCase and 10-fold amplification of human Alu sequences. We are presently cloning DNA from an amplified secondary transformant to isolate human genomic clones for the UMP synthase gene using human Alu sequences as markers for the gene. We have isolated a series of CHO mutants in UMP synthase: a) deficient in UMP synthase enzyme activity; b) revertants of the deficient mutants; and c) mutants with amplified UMP synthase enzyme activity. Analysis of a series of CHO mutants deficient in UMP synthase using Southern blots with a rat cDNA probe (kindly provided by D. Parker Suttle) suggests that no gross rearrangements or deletions are responsible for the enzyme deficiencies. CHO cells selected for resistance to azauridine and pyrazofurin have 35-fold elevated levels of UMP synthase enzyme activity with 10-fold UMP synthase gene amplification and 10-fold elevated UMP synthase mRNA levels. We are presently analyzing UMP synthase mRNA levels in the UMP synthase mutants and determining if there is transcriptional regulation of the UMP synthase gene.

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50 INFLUENCE OF METHOTREXATE ON PURINE AND PYRIMIDINE POOLS AND ON CELL PHASE DISTRIBUTION OF CULTURED HUMAN LYMPHOBLASTS

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Human leukemic lymphoblastic cell lines (Molt-4, Raji) were treated for 24 hrs with 0.2 µM Methotrexate (MTX). After 12 hrs, intracellular dCTP and dTTP concentrations decreased to 20% of the values of untreated cells. We noted a significant increase of UMP, UTP and CTP concentrations under these conditions. At the same time dATP and dGTP levels dropped to 30% and 50%, respectively. Accumulation of cells in G1 phase and decrease of cells in G2+M phase (to almost zero) was observed, after treatment with 0.2 µMMTX. The effect of 0.02 µM MTX on intracellular purine and pyrimidine concentrations was less pronounced, than that of 0.2 µM MTX, except for dCTP. In contrast to a decrease of dCTP, observed in experiments with 0.2 µM MTX, an increase of dCTP was measured in experiments with 0.02 µM MTX.
Cell cycle phase distribution studies with 0.02 µM MTX, revealed an accumulation of cells in early S phase and decrease of cells in G1 phase and G2+M phase.

51 DIHYDROTHYMINE DEHYDROGENASE DEFICIENCY IN A FAMILY LEADING TO ELEVATED LEVELS OF URACIL AND THYMINE.

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Recently we have reported about a seven year old girl, the second child of non-consanguineous parents, with dihydrothymine dehydrogenase deficiency (1). In this report we present additional data of studies on the family of the child. The mother and younger brother of the patient showed elevated uracil and thymine concentrations in urine and serum. The measured uracil and thymine concentrations of these two members of the family are comparible to those of the patient. The grandmother, father and older sister of the patient had uracil and thymine concentrations between the values of controls and of the patient.
In fibroblasts the activity of dihydrothymine dehydrogenase was determined, as previously described (1). The results demonstrated that the mother and brother have a complete dihydrothymine dehydrogenase deficiency, just as was described for the patient (1). A partial deficiency of this enzyme was found in the fibroblasts of the father. For the sister and grandmother such a partial enzyme deficiency might be expected, but has still to be proven.

1. J.A.J.M. Bakkeren, et al, Clin.Chim.Acta 140 (1984), 247-256.

52 INCREASED AVAILABILITY OF PHOSPHORIBOSYL PYROPHOSPHATE AS THE BASIS FOR ENHANCED 6-MERCAPTOPURINE INCORPORATION BY METHOTREXATE, IN CULTURED HUMAN LYMPHOBLASTS.

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In this study we have examined the inhibition of purine de novo synthesis by methotrexate (MTX), with concommitant increase of intracellular PRPP concentrations and enhancement of 6-mercaptopurine (6MP) incorporation. We found a significant elevation of PRPP concentrations in three cultured human leukemic cell lines (Molt-4, Raji, KM3), treated with 0.2 µM and 0.02 µM MTX, respectively. The maximal increase of the intracellular PRPP concentrations occured after distinct time intervals, dependent on both the MTX concentration and the cell type used.
During incubation with MTX, samples were taken at various time points and the cells were studied on the availability to incorporate [8-^{14}C] -6MP. Enhanced 6MP incorporation was observed in time after treatment. At the time points measured, the enhancement of 6MP incorporation correlated well with the increased PRPP levels.

53 IMBALANCE IN NUCLEOTIDE POOLS OF MYELOID LEUKEMIA CELLS AND HL-60 CELLS: CORRELATION WITH CELL CYCLE PHASE, CELL PROLIFERATION AND DIFFERENTIATION;

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Peripheral blood myelocytes from AML or CML patients as well as HL-60 cell-line cells show an imbalance in the nucleotide pool compared with normal peripheral neutrophils. This imbalance includes a decreased ratio of purine:pyrimidine nucleotides, and an increase in the relative concentrations together with an altered composition of the UDP-sugars. First, we studied the relation between this imbalance and the cell cycle. HL-60 cells were separated into fractions enriched in G1, S, or G2+M phase by elutriation centrifugation. The amount of nucleotides per cell differed during the cell-cycle (G2+M>S>G1), but the imbalance was found in all phases. Next, we studied the relation between the imbalance and the proliferation and/or immaturity of the cells. HL-60 cells were induced to myeloid differentation with DMSO: after 3 days of incubation proliferation stopped and after 6 days 70-90% of the cells reduced NBT upon stimulation with PMA. The nucleotide content and the imbalance diminished during differentiation, but this was also observed in non-induced non-proliferation HL-60 cells (negative NBT test). However, only DMSO-induced HL-60 cells showed a decreased UDP-sugar content, with relatively more UDP-glucose (as do normal peripheral neutrophils). We conclude, that the imbalance in the nucleotide pool of HL-60 and myeloid leukemia cells is largely related to proliferation, without differences between cell phases, whereas the changes in the UDP-sugars are associated with the immature and/or transformed character of the cells.

54 IMMUNOHISTOCHEMICAL DEMONSTRATION OF ADA AND ADCP ON THE CELL SURFACE OF HUMAN T-LYMPHOID CELLS.

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ADA plays an important role in the development of the immune system and the enzyme is probably associated with T-lymphocyte differentiation. ADA has been shown to exist in different molecular weight forms. In man the major forms are the red cell ADA or ADA-S and the tissue specific ADA or ADA-L. ADA-S can be converted to ADA-L by complexing with ADCP. Intact human peripheral T lymphocytes and human thymocytes were first incubated with rabbit anti-ADA and anti-ADCP antisera, followed by incubation with FITC-labeled swine anti-rabbit IgG. Since only cell surface ADA and ADCP would be exposed to the antisera, fluorescence indicates ADA and ADCP expression on the outer cell membrane. Labeled cells were analyzed by fluorescence microscopy and FACS analysis. ADCP is present at the cell membrane of about 50% of the T lymphocytes; 10-20% of the cells contain membrane-bound ADA. When these cells were incubated with a lysate of human erythrocytes (as source of ADA-S) about 50% of the cells were found to display ADA at the membrane. Human thymocyte suspensions contained 20-30% membrane-ADA positive cells and 10-20% membrane-ADCP positive cells. The physiological significance of ADA and ADCP expression at the extracellular surface of human T-lymphoid cells will be resolved.