73 MECHANISM OF THYMINELESS DEATH. M. Goulian, H. Ingraham, B. Tseng, L. Dickey, B. Bleile, and S. Neynaber. U. Cal. San Diego, La Jolla, CA, USA.

As a model for thymidylate deficiency we have studied cultured mammalian cells treated with methotrexate in the presence of a purine supplement (Hx). Associated with the fall in intracellular dTTP, there is greater than 1000-fold increase in dUTP, which is normally undetectable (<0.3 nM) (due to the normal dUTPase mechanism) but under these circumstances approaches, and can even exceed, dTTP. Since DNA polymerases do not distinguish between dUTP and dTTP, dUMP is incorporated into DNA, the first demonstration of uracil in eukaryotic DNA. Uracil is rapidly removed from the DNA by uracil-DNA glycosylase followed by excision repair of the resulting apyrimidinic sites. However, because of the high dUTP/dTTP ratio, repair results in re-insertion of uracil, leading to a futile cycle of removal and reinsertion, with the possibility (especially when dUTP exceeds dTTP) of progressively widening single-stranded gaps in the DNA. We have proposed that these hitherto unsuspected lesions may contribute to the toxicity of thymidylate deprivation. We have now been able to show that when dUTP is increased in cells, even in the presence of normal dTTP (produced by treating cells with dUrd in HAT medium), newly synthesized DNA is fragmented and the cells die, as with limiting thymidylate. Finally, the toxicity of low dTTP (caused by treating cells with methotrexate and Hx) is mitigated when dUTP is lowered (with glucosamine). These results strongly support an important role for elevated dUTP in thymineless death, through a mechanism of DNA damage resulting from uracil insertion and removal.

74 DEOXY-ATP (dATP) LEVELS IN ADENOSINE DEAMINASE (ADA) INHIBITED HUMAN PERIPHERAL BLOOD B AND T LYMPHOCYTES CULTURED IN DEOXYADENOSINE (AdR). Harry E. Gruber, Alan Cohen, Gary Firestein, Douglas Redelman, and Harry Bluestein University of California San Diego, La Jolla, Ca. USA.

In B and T lymphoblastoid lines, the differential sensitivity of T-cell lines to AdR is mediated by their preferential accumulation of the AdR metabolite, dATP. To determine whether this mechanism of AdR toxicity applies to normal human B and T cells activated from a resting state, we examined the effects of AdR on DNA synthesis and the accumulation of dATP in E-rosetting (E+) cells stimulated with PHA and in non-rosetting cells (E-) stimulated with Staph. aureus Cowan I. Cells were incubated for 40 hours in the presence or absence of 60 μM AdR and 1 μM 2′-deoxycoformycin (dCF), an ADA inhibitor. Inhibition of ^{3}H-thymidine uptake by AdR and dCF varied with the culture conditions. In the absence of dCF and AdR, neither E+ nor E- cells had dATP detectable by HPLC. However, ADA-inhibited E- cells incubated with AdR accumulated 101±6 pmol dATP/10^{6} viable cells, while comparably treated E+ cells accumulated 8±4 pmol dATP/10^{6} viable cells. In hereditary ADA deficiency, where T-cell function is more severely impaired than B, a mechanism other than the accumulation of dATP might be responsible.

75 INSERTION OF HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE (HPRT) cDNA INTO HUMAN BONE MARROW CELLS (BMC) BY A RETROVIRUS VECTOR. Harry E. Gruber, Theodore Friedmann, Kim D. Finley, Scott Katzman, Paul K. Laikind, Robert M. Hershberg, Lori A. Luchtman, J. Edwin Seegmiller and Douglas J. Jolly University of California San Diego, Departments of Medicine and Pediatrics, La Jolla, Ca. USA.

Cultured human BMC have been infected with a transmissible amphotrophic murine retroviral vector containing the human HPRT cDNA. Infection of the cultured cells and production of progeny vector has been determined by a colony formation assay in which culture medium from the infected cells is used to infect HPRT-deficient mouse fibroblasts. Successful marrow cell infection demonstrated by progeny virus production is indicated by formation of colonies of mouse cells resistant to hypoxanthine-aminopterin-thymidine (HAT) selective medium. Infected bulk marrow cells have been found to produce detectable progeny HPRT virus, and the conditions of infection including such variables as multiplicity of infection, time of infection, serum conditions, polybrene concentration and others are being optimized. Successful infection of a stem cell population has been demonstrated by efficient virus production from isolated granulocyte/monocyte colony forming units (CFU-GM) growing in semisolid (agar) medium.

76 GENETIC AND BIOCHEMICAL CHARACTERISTICS OF THREE DIFFERENT TYPES OF MUTANTS OF CHINESE HAMSTER OVARY CELLS AFFECTED IN ADENOSINE KINASE. Radhey S. Gupta and Kamal D. Mehta, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

In CHO cells, three different types of mutants affected in purine nucleoside salvage pathway enzyme adenosine kinase (AK) have been isolated. One class of mutants obtained at high frequency using various adenosine analogs (Class A) exhibit high degree of cross resistance to various C- as well as N- adenosine analogs. These mutants show greatly reduced phosphorylation of all adenosine analogs and cell extracts from these contain no measurable activity of AK. The second type of mutants (Class B) obtained using formycin A (a C- adenosine analog) exhibit increased cross resistance to various C- nucleosides (viz. 9-deazaadenosine, pyrazofurin, etc.),but their sensitivity towards various N- adenosine analogs was found to be unaltered. Cell extracts from these mutants also contained no measurable activity of AK. Reduced phosphorylation of C- adenosine analogs but not N- adenosine analogs by Class B mutants provides strong evidence that they contain a novel genetic lesion affecting AK that specifically affects the phosphorylation of C- nucleosides. The third type of mutants (Class C) selected using inosine analog formycin B, exhibit cross resistance and reduced phosphorylation of both C- as well as N- adenosine analogs. However, their degree of resistance was lower in comparison to the Class A mutants. Cell extracts from the Class C mutants contained between 50-100% of AK activity, but its affinity towards adenosine analogs was found to be altered in comparison to the wild-type enzyme.

77 PRODUCTION AND PROPERTIES OF MONOCLONAL ANTIBODIES AGAINST HUMAN ECTO-5′-NUCLEOTIDASE

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After immunization of mice with partially purified human placental 5′-nucleotidase hybrid cells were produced following the standard protocol. Culture supernatants were screened for the ability to inhibit enzyme activity. From 18 different hybrids showing significant inhibition 5 were established as clones. Properties of the MABs were studied in ascites fluid and in purified Ig-preparations. Inhibition of the MABs is directed against the enzyme in membrane bound and solubilized form and there is no tissue specificity. Similar inhibitory titers were observed with the MABs coated on plastic. The MABs belong to the IgG 1 and IgG 2b subclasses. Some of them show a marked temperature dependence in their inhibitory potency. They are not directed against the sugar moiety of the 5′-N molecule. In fluorescence studies with PBL a smaller subpopulation, compared to polyclonal antisera, shows a positive reaction with the MABs. The titers for optimal fluorescence are much lower than for inhibition. Results of flow-cytometric studies relating cell size to positive reaction with the MABs will be reported.

78 ECTOENZYMES OF NUCLEOTIDE METABOLISM ON HUMAN LYMPHOID CELLS

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In addition to the well studied 5′-nucleotidase other enzymes of nucleotide metabolism are characterized on the surface of human lymphoid cells, especially on lymphoblastoid B-cell-lines: An ATPase, an ADPase and a nucleosidediphosphate kinase. The true ectoenzyme nature of all three activities is established by various independent criteria. Typical properties such as substrate specificities, metal ion requirement and kinetic constants are determined. Treatment of intact lymphoblasts with sublytic concentrations of certain detergents induces membrane shedding. All three enzymes together with 5′-nucleotidase are enriched in the shed membrane fraction. All these ectoenzymes seem to follow a pattern of coordinated expression in lymphoblastoid B-cell-lines, with low or absent activities in lines derived directly from Burkitt′s lymphoma and high activities in lines established by EBV-infection of normal PBL. On the other hand ATPase, compared to 5′-nucleotidase is much less suitable as an enzyme marker for differential diagnosis in acute leukemias.