37 ROLE OF INTRACELLULAR DEOXYNUCLEOSIDE TRIPHOSPHATES LEVELS IN DNA DAMAGE IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES. Amos Cohen. Division of Immunology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

The effect of deoxynucleosides on single strand chromosomal breaks in peripheral blood lymphocytes (PBL) were measured both by the sucrose gradient nucleoid sedimentation method and by alkaline unwinding followed by fluorimetric measurement of etidium bromide binding to double stranded DNA. Extracellular deoxyadenosine (0.3-10 μM) in the presence of deoxycoformycin (1 μM) cause extensive DNA damage in PBL in a concentration dependent manner. The appearance of DNA damage following deoxyadenosine addition to PBL correlated with the accumulation of intracellular dATP levels. Extracellular deoxycytidine (50 μM) inhibited the accumulation of intracellular dATP from deoxyadenosine and in parallel prevented the DNA damage caused by deoxyadenosine in PBL. On the other hand, addition of extracellular deoxyguanosine (50 μM) or thymidine (50 μM) also completely prevented the DNA damage by deoxyadenosine but without interferring with dATP accumulation. Measurements of intracellular deoxynucleoside triphosphates levels in PBL show extremely low levels of dGTP, dCTP and TTP and higher levels of dATP. It is possible that the marked increase of intracellular dATP levels combined with the extremelylow levels of the other deoxynucleoside triphosphates interfer with DNA repair process in resting PBL resulting in the appearance of single stranded breaks. Thymocytes, which contain higher levels of deoxynucleoside triphosphates, did not show DNA damage in the presence of deoxyadenosine.

38 MORPHINE: SITES OF ACTION IN GUANOSINE NUCLEOSIDE PATHWAY. Major L. Cohn, Faye A. Eggerding, Antonio F. Machado and Stephan J. Cohn. Drew/UCLA School of Medicine. Department of Anesthesiology Research. Los Angeles, California USA

Though cyclic GMP (cGMP) has been strongly implicated in morphine's analgetic action, the relationship between pharmacologic activity of morphine and brain levels of cGMP is not yet well understood. Here, we investigated effects of morphine on cGMP metabolism. Brain slices were incubated in tonometer at 37°C with Krebs-Ringer bicarbonate/glucose buffer, pH 7.35, constant flow of O_2/CO_2 (20:5) and 2.90 x 10^{-1} mM of substrate cGMP or 5'-GMP. Sequentially withdrawn aliquots of incubation mixture were analyzed by HPLC. Control brain slices yielded primary metabolic products, GMP, guanosine (GUS), guanine, xanthine, and inosine. Accumulation of guanosine agreed with evidence demonstrating that nucleoside phosphorylase is rate limiting. Chromatographic findings of inosine from samples of human brain slices incubated with cGMP or 5'-GMP as substrate extended our earlier report of 5'-GMP reductase activity in rat brain. No uric acid was detected. Morphine (1.46 x 10^{-2} mM) significantly reduced rate of cGMP and 5'-GMP metabolisms resulting in latent GUS accumulation substantially increased over control values. Our data suggest that morphine alters purine metabolism by inhibiting cGMP phosphodiesterase and 5'-nucleotidase. This may ultimately assist in establishing both biological significance of cGMP metabolites and multiple actions of morphine on the central nervous system. Supported by NIH/MBRS Grant RR 0809-12.

39 PARTIAL PURIFICATION OF SOLUBLE 5'-NUCLEOTIDASE FROM RABBIT HEART. Albert R. Collinson, Janet S. Zisk, and John M. Lowenstein, Dept. of Biochemistry, Brandeis University, Waltham, MA 02254

Cytosolic 5'-nucleotidase from rabbit heart has been purified approximately 40-fold to a specific activity of 0.6 μmol/mg protein/min. At this stage of purification, the enzyme is free of other phosphatases and is stable to repeated cycles of freezing and thawing over a 3 months period. Soluble 5'-nucleotidase has an absolute requirement for divalent metal ions and is activated by ATP, GTP, and ADP. Activation by these nucleotides causes a shift from a sigmoidal to a hyperbolic substrate saturation curve. ATP (1 mM) increases the K_{app} of the enzyme for AMP about 5-fold. Activation by GTP is similar. Activation by ADP also increases K_{app}, but high concentrations of ADP cause a decrease in V_{max}. AMP is the preferred substrate in terms of V_{max}/K_{app}, with AMP>UMP>GMP>IMP. Nucleoside monophosphates other than AMP are inhibitory at high substrate concentrations. The fully activated enzyme exhibits a K_{app} for AMP of 2.5 mM. The enzyme purified by us has properties different from the soluble liver enzyme described by Van Den Berghe et al. (Biochem. J. (1977) 162, 611) and from the plasma membrane enzyme from rat heart described by Naito & Lowenstein (Biochem. (1981) 20, 5188) with respect to both substrate specificity and regulatory properties.

40 ELEVATED ERYTHROCYTE ADENOSINE DEAMINASE (ADA) ACTIVITY IN ACQUIRED IMMUNODEFICIENCY DISEASE (AIDS) Morton J. Cowan and Kenneth J. Widder, University of California, School of Medicine, Department of Pediatrics, San Francisco and Molecular Biosystems, Inc, San Diego, CA USA

AIDS is an often fatal disease thought to be caused by a retrovirus (LAV) frequently resulting in malignancy such as Kaposi's sarcoma (KS) and/or opportunistic infection (OI). One AIDS related disease is lymphadenopathy syndrome (LAS) which may progress to KS or OI. Because the immune deficiency in AIDS is similar to that in some purine enzyme deficiencies we measured erythrocyte ADA and purine nucleoside phosphorylase (PNP) activities (nm/mgHg/hr) in patients with AIDS, heterosexual controls (CONT) and a high risk asymptomatic population (RISK). ADA was significantly elevated in the AIDS patients and in 5/11 RISK patients while PNP was normal (mean ± SD):

	CONT	KS	OI	KS/OI	LAS	RISK
ADA	25±10	36±8	34±8	53±12	43±11	33±13

Anti-LAV correlated with ADA in the RISK patients while T-cell immunity was relatively unaffected.

	ADA	ANTI-LAV	T4/T8	PWM(cpm)
RISK: HIGH ADA (5)	45±4	4/4	1.1±0.4	14145±5311
NL ADA (6)	22±4	0/4	1.6±0.4	17091±5643
AIDS (20)	40±11	---	0.2±0.1	1136±1829
CONT (16)	25±10	---	2.2±0.4	10039±9899

Increased ADA appears to be a diagnostic marker of AIDS and may be useful as a prognostic indicator of disease progression in asymptomatic high risk individuals.

41 RESTORATION OF IN VITRO FUNCTION OF ADENOSINE DEAMINASE (ADA) DEFICIENT LYMPHOCYTES BY INTERLEUKIN-2 (IL-2)

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We evaluated the effects of recombinant DNA IL-2, a T-cell lymphokine which is essential for normal immune function on the responses to mitogen and alloantigen of peripheral blood mononuclear cells (PBMC) from 3 ADA deficient patients. There was significant enhancement by IL-2 of mitogenic responses to phytohemagglutinin (PHA), pokeweed mitogen (PWM) and alloantigen (MLR) in all 3 patients. Patients with ADA positive severe combined immunodeficiency disease showed no responses to IL-2:

	PHA(+IL-2)	PWM(+IL-2)	MLR(+IL-2)
Control	40255(56566)	4969(4780)	16505(15623)
ADA def #1	606(23449)	3163(8151)	218(1526)
ADA def #2	1190(20354)	2290(6086)	--
ADA def #3	282(23182)	--	--

To determine the responsive cell population we measured T3, T4 and T8 expression in PBMC exposed to PHA ± IL-2. Normal PBMC respond with increased T3 cells but no change in T4 or T8 expression. The ADA deficient PBMC incubated with an ADA inhibitor, deoxyadenosine and PHA cannot be salvaged by IL-2. These results demonstrate a unique feature of the immunodeficiency in ADA deficiency and provide further evidence that purine metabolism is distinct in lymphocyte subsets. Also, the standard *in vitro* cell model of ADA deficiency may be significantly limited in its use in understanding the pathogenesis of this disease.

42 EXPRESSION DEFECTS OF MUTANT HUMAN ADENOSINE DEAMINASE. Peter E. Daddona, Stewart H. Orkin, and William N. Kelley, University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor, MI, USA.

A severe deficiency of adenosine deaminase (ADA) is associated with an autosomal recessive form of severe combined immunodeficiency disease, while subjects with partial enzyme activity are immunocompetent. To further our understanding of ADA deficiency, specific mRNA levels and rates of protein turnover were determined in partial ADA deficient cells and enzyme expression of cloned human ADA cDNA was assessed. Genetic expression of ADA in cultured B lymphoblast cell lines from 4 partially (5-50% activity) ADA deficient subjects showed normal to 2-fold normal ADA mRNA levels (Northern blot). Among these cell lines enzyme synthesis varied from 165% to 15% of normal. ADA degradation rates were 1.5 to almost 3 times faster than normal, consistent with the complete absence of the enzyme in patient erythrocytes. Cloned normal human ADA cDNA inserted into an SV_{40} based expression vector was shown to generate human ADA activity in monkey kidney (COS) host cells (7-fold over host). An unexpected finding was the identification of another normal human ADA cDNA construct which failed to produce human ADA activity or immunoreactive protein upon transfection. DNA sequence analysis revealed a single nucleotide substitution within this clone that altered the predicted sequence of ADA protein at amino acid 50.

Thus, our studies define transcriptional, translational and post-translational defects in partial ADA deficiency and identify a point mutation in ADA cDNA which mimics the phenotype expressed in severe ADA deficiency.