229 ABERRANT ACTIVITY OF ADENOSINE DEAMINASE IN THE DIAMOND BLACKFAN SYNDROME

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Diamond Blackfan syndrome (DBS) is a severe congenital hypoplastic anaemia which may be accompanied by an up to 5 fold increase in red cell adenosine deaminase (ADA) activity (1,2). In a study of more than 10 patients with DBS we have found that the correlation between this disorder and elevated red cell ADA activity is not complete. However, we have observed that elevated ADA activity may be present in leukocytes and cultured fibroblasts of some DBS patients, suggesting that the abnormality can extend beyond the erythroid cells. The ADA protein in red cells of patients displaying hyperactivity is apparently normal as judged by a variety of experimental procedures. In a few cases a familial clustering of the anomolous ADA activity is evident, supporting previous observations (1) and indicating that the phenomenon may have a genetical basis. From our studies we can be sure that this hyperactivity is not due to allelic variation at the ADA structural locus and is more likely to result from a trans acting regulatory mechanism.

1. Glader, B.E., Backer, K., and Diamond, L.K. (1983) N. Engl. J. Med. 309, 1486-90.
2. Whitehouse, D.B., Hopkinson, D.A., and Evans, D.I.K. (1984) Lancet II, 1398-99.

230 ADENOSINE DEAMINASE DEFICIENCY AND CHONDRO-OSSEOUS DYSPLASIA:

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Purine metabolism was investigated in an in vitro model of adenosine deaminase (ADA) deficiency in rabbit epiphyseal growth plate (EGP) and articular cartilage (AC) to elucidate the biochemical basis for the poorly understood chondro-osseous dysplasia that occurs with ADA deficiency.

Deoxyadenosine (dado) in the presence of EHNA, an ADA inhibitor, is toxic in a dose dependent fashion to EGP in organ culture as determined by LDH release. Dado or EHNA alone as well as adenosine, homocysteine thiolactone and deoxyguanosine alone or in combination are not toxic. The model is not toxic to mature AC.

The combination of 100 µM dado and 5 µM EHNA causes decreased radiolabeled thymidine, glycine and SO_4 incorporation (92%, 64%, and 36%, respectively) by EGP cartilage in organ culture for 5 days but does not affect isotope incorporation in AC.

Incubations of released chondrocytes in the model causes deoxyATP accumulation in EGP and AC cells at 48 hours. ATP concentrations are undetectable at 48 hours in EGP cells but 1.4 ± 0.4 nmol/10^6 AC cells.

These data demonstrate a selective toxic effect of the combination of dado and an ADA inhibitor to EGP cartilage and suggest that the chondro-osseous dysplasia of ADA deficiency is the consequence of the deficiency state. ADA deficiency may cause altered chondrocyte function and viability by ATP depletion.

231 ECTO-NUCLEOSIDE TRIPHOSPHATE PYROPHOSPHOHYDROLASE ACTIVITY AND CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTAL DEPOSITION DISEASE.

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Elevated levels of nucleoside triphosphate pyrophosphohydrolase (ENP) (NTP→NMP+PPi) are present in cartilage extracts of patients with calcium pyrophosphate (PPi) crystal deposition disease (CPPD) (Tenenbaum, Arth Rheum 24:492, 1981).

PPi is generated from 1 mM ATP in dog articular cartilage organ culture (800 ± 300 pmol/hr/mg wet wt, n=11. The specific activity of (^{32}P)PPi produced from 1 mM gamma-(^{32}P)ATP is 97% that of the labeled ATP. AMPCP, EHNA, and dipyridamole do not alter PPi generation in this system. No PPi is generated when adenosine, AMP or ADP are substituted for ATP.

ENP activities on cultured human skin fibroblasts from 24 CPPD subjects are elevated compared to 13 oestoarthritic and 7 normal controls ($p<0.002$, Wilcoxon's rank sum) with higher values for 13 sporadic compare to 11 familial cases of CPPD. No differences exist for ecto-5'nucleotidase or pyrophosphatase. Intracellular PPi is higher for CPPD fibroblasts ($p<0.0002$) and correlates with ENP activity ($r_S=0.49$, $<p.0.05$, Spearman's rank correlate).

These data demonstrate the in vitro generation of PPi in cartilage in the presence of ATP is entirely due to ENP activity. The expression and variation of ENP activity and intracellular PPi levels in nonarticular human cells support the hypothesis of a generalized metabolic defect in CPPD.

232 PHOSPHORYLATION OF PURINE DEOXYNUCLEOSIDES IN HUMAN T- AND B-LYMPHOBLASTS

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In B-lymphoblast, deoxyadenosine (dAdo) is phosphorylated by adenosine kinase (AK), deoxycytidine kinase (dCK) and deoxyguanosine kinase (dGK), and deoxyguanosine (dGuo) is phosphorylated by dGK and dCK. Fractionation of nucleoside kinases by DEAE-cellulose chromatagraphy showed the existence of an extra nucleoside kinase in T-lymphoblast. This enzyme, T-lymphoblast-specific nucleoside kinase (TSK) phosphorylates dAdo, dGuo, deoxycytidine and arabinocytidine, similar to dCK, but the respective kinetic properties are quite different. TSK is also different from dCK in the molecular weights (TSK, 26,500; dCK, 56,000), in the pI values (TSK, pH 8.2; dCK, pH 4.8), in the phosphate donor specificities, and in the dCTP inhibition. The Km values of TSK for dAdo and dGuo, 11.3 and 24.0 µM, respectively, are much smaller (about 1/10) than those of dCK. Further, there is no inhibitor which objects TSK at physiological concentration, while AK, dCK and dGK are inhibited strongly by adenosine, dCTP and dGTP, repectively. Therefore, the phosphorylation rates of dAdo and dGuo in TSK containing T-lymphoblast must be higher than the rates in B-lymphoblast. The inhibition studies in T and B cell extracts using the inhibitors of AK, dCK and dGK, suggest that dAdo and dGuo are phosphorylated mostly by TSK in T-lymphoblast.

233 COMPARISON OF SURFACE DIFFERENTIATION ANTIGENS BETWEEN SEPARATED HGPRT-POSITIVE AND NEGATIVE T-CELL POPULATIONS FROM TWO LESCH-NYHAN HETEROZYGOTES

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Peripheral T-lymphocyte populations from two Lesch-Nyhan heterozygotes were found to consist of 95% hypoxanthine-guanine phosphoribosyltransferase (HGPRT)-positive and 5% negative cells by the culture of the T-cells with 6-thioguanine. The T-cells frome each heterozygote were cultured in the conditions in which either enzyme-positive (azaserine-hypoxanthine medium) or negative (6-thioguanine medium) cells selectively proliferate. After two weeks, each separated T-cell subpopulation was considered to be pure with regard to the enzyme expression. Bewteen the enzyme-positive and negative subpopulations, percentages of OKT3, OKT4 or OKT8-positive T-cells were compared. The fact that only small percentage of peripheral T-cells are HGPRT-negative in the heterozygotes suggests that in vivo differentiation of HGPRT-negative cells is disturbed in the heterozygotes. Our data have shown that both enzyme-positive and negative T-cell subpopulations from each heterozygote possessed equivalent percentages of OKT4, OKT8 and OKT3-positive cells. These results suggest that, in the heterozygotes, growth or differentiation of HGPRT-nnegative lympoid cells of T-cell linkage was disturbed before OKT4 and OKT8-bearing thymocyte subpopulations separate from each other.

234 2-CHLOROADENOSINE IS PHOSPHORYLATED AND INCREASES THE PRODUCTION OF HYPOXANTHINE IN HUMAN CELLLS

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2-Chloroadenosine (ClAdo) has been considered not to be phosphorylated by human cells. We cultured a human B cell line WI-L2 with this adenosine analog and found that human cells are able to phosphorylate ClAdo. The human cells were indeed killed by the drug. Since adenosine kinase negative variant of WI-L2 cells phosphorylated ClAdo at a slower rate than the parenteral cells, and the former were less sensitive than the latter to ClAdo, adenosine kinase was considered to participate in the phosphorylation and cytotoxicity. However, ClAdo was phosphorylated, although to minor extent, by adenosine kinase negative cells, while they were not able to phosphorylate MMPR, suggesting that other enzyme(s), in addition to adenosine kinase, also participate in the phosphorylation. We determined the rate of the production of hypoxanthine using HGPRT-negative WI-L2. When ClAdo was added to the medium, the human cells synthesized hypoxanthine more rapidly than control cultures containing no ClAdo. We found that ClAdo, at low concentrations, increased the level of IMP within the cells, and, at higher concentrations, decreased the level of ATP. These data suggest that ClAdo, by consuming ATP, increases the production of IMP and then hypoxanthine. There are numbers of reactions within the body that consume ATP, and acceleration of some of those reactions may be associated with the mechanisms of human hyperuricemia.