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AMP DEAMINASE AND THYMIDINE KINASE DEFICIENCIES IN A CLONALLY ISOLATED DERIVATIVE OF MOUSE S49 CELLS

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A mutant clone of murine S49 cells, DTB6, was isolated in a single step from mutagenized wild type cells by virtue of its resistance to 1 mM thymidine and 1mM dibutyryl cyclic AMP. In comparative growth rate experiments, DTB6 cells were considerably less sensitive than parental cells to the growth inhibitory effects of thymidine and thymidine analogs, but surprisingly were equally sensitive to dibutyryl cyclic AMP. Conversely, DTB6 cells were much more sensitive to the cytotoxic effects of either adenine or adenosine-EHNA. This supersensitivity of DTB6 cells to growth inhibition by adenine could be ameliorated by the addition of hypoxanthine to the culture medium. The complex growth phenotype of the mutant cells could be attributed to a 60% deficiency in AMP deaminase activity and a complete absence of thymidine kinase activity in the mutant cells. Revertants of DTB6 cells possessed wild type levels of AMP deaminase activity but remained deficient in thymidine kinase activity, while another revertant of DTB6 cells expressed 11% of the wild type thymidine kinase level but did not perceptibly change its AMP deaminase activity. The ability to isolate single step mutants with two seemingly independent biochemical abnormalities raises the speculation that there may be some link between cellular functions responsible for purine nucleotide and thymidine metabolism.

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PARTIAL PURIFICATION OF THE NUCLEOSIDE TRANSPORTER FROM HUMAN ERYTHROCYTES.

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The nucleoside transporter of human erythrocytes can be identified by photolabelling with radiolabelled nitrobenzylthioinosine, a potent high affinity inhibitor of nucleoside transport in human erythrocytes and some somatic cells. The nucleoside transporter migrates as a broad band on SDS polyacrylamide gels with a molecular weight between 45,000 and 65,000 daltons. This band width probably reflects heterogeneous glycosylation. The nucleoside transporter from human erythrocytes has been partially purified by a combination of high pH washes to remove extrinsic proteins followed by ion exchange chromotography on DEAE-cellulose. In this fashion, the nucleoside transporter has been purified approximately 10 to 20 fold from human erythrocyte ghosts. However, the nucleoside transporter preparation was contaminated by the glucose transporter. Efforts are currently underway to generate proteolytic fragments of the nucleoside transporter that are distinct and separable from the glucose transporter and to separate the glucose transporter from the nucleoside transporter by immunoaffinity chromatographic techniques. The focus of these studies is to generate a sufficiently pure preparation of nucleoside transporter that can be used to obtain amino acid sequence or to generate antibodies that can be subsequently exploited for the isolation of molecular clones encoding the human nucleoside transport system.

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PURINE DEGRADATION IN ISCHEMIC AND NON-ISCHEMIC CONTRACTING MUSCLES OF RATS

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Degradation of purine nucleotides was evaluated in different types of rat skeletal muscle during ischemic and non-ischemic contraction. Extensor digitorum longus (EDL, fast) and soleus (slow) muscles were stimulated electrically via the sciatic nerve (5 Hz, 10 min). Under non-ischemic condition, the concentrations of IMP, inosine, adenosine, and hypoxanthine increased in EDL muscles but not in soleus muscles during stimulation. Under ischemic condition, these metabolites increased in both EDL and soleus muscles and the changes in concentrations of IMP and inosine were greater in ischemic EDL muscles. The increase in inosine had a strong positive correlation with that in IMP in ischemic EDL and soleus muscles, but the ratio, Δinosine/ΔIMP was smaller in EDL muscles. The increase in adenosine under ischemic condition was not significantly different between the two muscles. These findings suggest that ischemia enhances overall degradation of purine nucleotides in contracting fast and slow muscles, and that although the degradation of adenine nucleotides to IMP is greater in fast muscles than in slow muscles, the relative degradation rate of IMP to inosine with respect to the intramuscular IMP concentration is rather smaller in fast muscles.

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LESCH-NYHAN SYNDROME: REDUCED AMINO ACID CONCENTRATIONS IN CSF AND BRAIN

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In marked HPRT deficiency, hypoxanthine accumulation in CSF is greater than in plasma. The extent of other severe biochemical disturbance could provide further evidence of the mechanisms underlying the severe neural dysfunction.

A generalised reduction of the concentrations of 13 amino acids was found in CSF (mean percentage of control 25.5 range 12-48%) and in 5 regions of brain, 34, 21-75% from the same patient, D, who had normal plasma and urine amino acid levels. A neurotransmitter metabolite, 5-HIAA, in CSF from patient D was slightly reduced but concentrations of metabolites of noradrenaline and dopamine were normal. Another patient had normal amino acid concentrations in CSF, plasma and urine.

Our results on patient D have provided more evidence of the marked reductions in brain amino acid concentrations which have been found in 5 other brains from patients with the Lesch-Nyhan syndrome (Neuropaediatrics 13, 130). Our findings in CSF suggest that such reductions can exist during life and from the normal results in plasma and urine are not due to undernutrition.

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A ROLE OF INTERLEUKIN 1(IL-1) IN CRYSTAL INDUCED ARTHRITIS

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Monosodium urate (MSU) crystal is known to play an important role in the pathogenesis of gouty arthritis. Recently MSU has been proposed to be one of the potent IL-1 stimulators from adherent mononuclear cells (MNC). We intend to clarify the role of IL-1 in the pathogenesis of gouty arthritis. Adherent MNCs obtained from peripheral blood of gouty patient and cloned synovial cells were exposed to either MSU, Hydroxyapatite (HA), or calcium pyrophosphate dihydrate (CPPD). Cloned synovial cells were biopsied from patients with gout or osteoarthritis under arthroscopy and classified into three types, i.e. dendritic cells (DC) macrophage-like cells (MC), and fibroblast-like cells (FC). IL-1 production increased with a stimulation by crystals in a dose-dependent fashion when MNCs and synovial adherent cells were used. IL-1 activity was also detected in the culture supernatants of three types of cloned synovial cells stimulated with any of the crystals tested. These results suggested that IL-1 may be an important mediator of crystal induced arthritis. This is the first report of IL-1 release upon stimulation of cloned synovial cells with crystals.

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DIVERGENT EFFECTS OF BREDININ ON HUMAN T CELL SUBSETS.

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Bredinin (4-carbamoyl-1- β-D-ribofuranosylimidazolium-5-olate) is an adenosine analog with immunosuppressive activities. This compound is phosphorylated by adenosine kinase to bredinin-5'-monophosphate which inhibits IMP dehydrogenase and depletes the cellular guanine nucleotides. Similarly, structurally related nucleoside analogs tiazofurin, ribavirin, and selenazofurin inhibit IMP dehydrogenase and exhibit potent antiviral activity. Since bredinin effectively kills the proliferating mammalian cells, bredinin has been clinically used as an immunosuppressive agent to prevent rejection reactions after kidney transplantation and to treat rheumatoid arthritis (RA). We have analyzed the effects of bredinin toward the activated T cell subsets in vitro. Peripheral blood mononuclear lymphocytes (PBLs) were obtained from 10 healthy subjects and 11 RA patients. PBLs were cultured with PHA in the presence or absence of bredinin for 5 days, and T4/T8 ratio was measured by flowcytometry. Bredinin treated T lymphocytes showed increased T4/T8 ratio in a dose dependent manner, showing that suppressor/cytotoxic T cells are more susceptible to bredinin than helper/inducer T cells. On the other hand, no significant changes were observed when cells were cultured with azidothymidine or deoxyguanosine. These results suggest that bredinin has a unique divergent effects on T lymphocyte subsets, and could be an useful therapeutic reagent for diseases with abnormal immune regulation.