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IMMUNOLOGICAL AND METABOLIC RECONSTRUCTION FOLLOWING SUCCESSFUL BONE MARROW TRANSPLANTATION FROM A HLA-IDENTICAL SIBLING IN AN INFANT WITH ADENOSINE DEAMINASE DEFICIENCY AND SEVERE COMBINED IMMUNODEFICIENCY

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Adenosine deaminase (ADA) activity and immunological functions following successful bone marrow transplantation (BMT) was evaluated for 3 years in a 3-year-old boy with ADA deficiency and severe combined immunodeficiency (SCID) who was diagnosed at the age of 1 month and received BMTs three times during the following 3 months from a HLA-identical brother. On 12th day of posttransplantation, ADA activities of lymphocytes and red cells, which were below one-tenth of the normal levels, began to increase and reached within the normal level on the 19th day. Simultaneously, dATP in red cells and dAdo in urine reduced significantly. The persistent chimerism of donor red cells disappeared spontaneously 2 years later, when ADA activity in red cells had decreased further to undetectable level. On the other hand, B-cell functions began to recover about one month after transplantation. T-lymphocytes with the surface markers of OK3, OK4, Leu10 and Leu7 increased in number within the normal levels, although T-lymphocytes with positive OKT8 antigen delayed to appear until about 2 months later. The in vitro responsiveness to PHA and Con-A mitogens started to recover slowly on about 3rd month after 3rd BMT. The patient is still quite healthy and develops very well without any GVHD.

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PARTIAL CHARACTERIZATION OF PHOSPHORIBOSYL-PYRAPHOSPHATE SYNTHETASE cDNA CLONES FROM HUMAN TESTIS. Taizo Iizasa, Masanori Taira, Kazumi Yamada and Masamitsu Tatibana. Chiba University School of Medicine, Department of Biochemistry, Inohana, Chiba 280, Japan.

Phosphoribosylpyrophosphate (PRPP) synthetase is a key enzyme for de novo and salvage syntheses of nucleotides. PRPP synthetase activity is distributed in almost all tissues. cDNA cloning of rat PRPP synthetase showed the existence of two distinct types of subunits, referred to as PRS I and II (JBC 262; 14867, '87). Northern blot analysis of RNAs from various mammalian tissues, using rat PRS I and PRS II cDNA as probes, showed that the expression of each gene is regulated in a tissue-specific manner: mRNAs of rat PRS I and/or PRS II were highly detected in brain, adrenal gland, spleen, lung, thymus, adipose tissue, and testis. The sizes of mRNAs for PRS I and II were 2.3 kb and 3.7 kb (2.7 kb for humans), respectively. In addition, in testes of rat, mouse, and humans, a unique PRS I-related 1.4 kb transcript was found, together with regular PRS I and II mRNAs. To characterize human PRS I and II mRNA as well as testis-specific one, the cDNA library was constructed in lambda phage vector λ gt 10 from human testicular poly(A)⁺RNA and 1.35×10^6 recombinants were screened with rat PRS I and II cDNAs as probes. Two types of clones were obtained: with PRS I cDNA probe, clones with a 1.2 or 1.1 kb insert and with PRS II probe, clones with a 2.4, 2.3, 2.1, or 1.8 kb insert. The former group covered the full coding region and may correspond to the specific 1.4 kb transcript and the latter to the PRS II transcript.

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RECOVERY OF THE ACTIVITIES OF IMP DEHYDROGENASE AND GMP SYNTHASE AFTER TREATMENT WITH TIAZOFURIN AND ACIVICIN IN HEPATOMA CELLS IN VITRO

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Tiazofurin, a C-nucleoside, and acivicin, a glutamine antagonist, are targeting against de novo guanylate synthesis and are under clinical trials as anticancer drugs. To elucidate the mechanisms of the recovery of the activities of the target enzymes after inhibition by these drugs, hepatoma 3924A cells were incubated either with 100 μ M tiazofurin or 10 μ M acivicin for three hours and the enzyme activities in cells after replacement with fresh medium were measured. IMP dehydrogenase, inhibited by 86% with tiazofurin, recovered to the control level within two hours. The addition of 6.7 μ M cycloheximide did not affect this recovery. GMP synthase activity, which acivicin completely inhibited, recovered to 82% within 36 hours and cycloheximide blocked this return. These results suggest that in tissue culture system the recovery of GMP synthase activity after acivicin treatment is due to the newly synthesized enzyme protein. By contrast, under these in vitro conditions, inhibition of IMP dehydrogenase by tiazofurin is released by other mechanisms, e.g., dissociation of the drug from the enzyme or degradation of the drug. The applicability of the observations obtained in this in vitro system to animals and in human chemotherapy is under investigation. (Supported by Outstanding Investigator Grant CA-42510 to G.W.)

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ISOZYME SHIFT OF ADENYLOSUCCINATE SYNTHASE IN RATS AND HUMAN NEOPLASMS

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The distribution of two isozymes of adenylosuccinate synthase was measured in rat transplantable tumors, human tumors, and their corresponding normal tissues. The two isozymes, the acidic and basic enzymes, were separated by DEAE cellulose column and the proportions of each fraction were quantitatively compared. In rat liver, the activity was distributed 47% in the passed fraction of basic enzyme and 53% in the adsorbed fraction of acidic enzyme. In hepatoma 3924A, however, 99% of activity was found in the adsorbed fraction. Adenylosuccinate synthase in rat skeletal muscle was entirely basic enzyme, whereas sarcoma consisted of 99% acidic enzyme and 1% basic enzyme. The distributions of acidic enzyme in human liver and colon mucosa were 24% and 62%, respectively. On the other hand, the values increased in human hepatocellular carcinoma and colon carcinoma to 86% and 96%, respectively. These results suggest that the isozyme pattern of adenylosuccinate synthase shifts into a predominance of the acidic enzyme in malignant tissues. The increase in adenylosuccinate synthase activity in neoplastic tissues reported earlier (Jackson, R.C., Morris, H.P., and Weber, G., Cancer Res. 37: 3057, 1977) and the shift to the acidic isozyme with a lower K_m for IMP indicate an increased capacity for adenine nucleotide biosynthesis in cancer cells. These observations are in line with the isozyme shift identified for other enzymes in hepatomas (Weber, G., Cancer Res. 43: 346, 1983). (Supported by Outstanding Investigator Grant CA-42510 to G.W.)

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PARADOXICAL EFFECTS OF GLUCAGON ON DE NOVO PURINE SYNTHESIS IN RAT LIVER

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In the prereplicative period of regenerating rat liver after a 70% hepatectomy, "the rate of de novo purine synthesis" ("purine synthesis") increases by 2.4-fold in parallel with plasma glucagon (2.5-fold), hepatic cyclic AMP (cAMP) (2-fold) in the second peak, 5-phosphoribosyl-1-pyrophosphate (PRPP) (3.0-fold) and specific activity of amidophosphoribosyltransferase (ATase) (1.8-fold) (1). Based on these correlations, it was hypothesized that glucagon promotes liver regeneration by increasing "purine synthesis". To test this hypothesis, the effect of infusion or bolus administration of glucagon or dibutyryl cAMP (Bt₂cAMP) was studied in rat liver in regard to "purine synthesis", ATase activity, concentrations of cAMP, PRPP and purine ribonucleotides. "Purine synthesis" increased (3.5-fold) by infusion for 12 hours (2), but was transiently inhibited by bolus administration (0.5-fold), in spite of increased cAMP (5- vs. 50-fold) and PRPP (1.5- vs. 2.4-fold) concentrations in a dose-dependent and time-dependent manner in both conditions (3). All the other factors were unchanged. These paradoxical effects of glucagon or Bt₂cAMP on "purine synthesis" suggest that the mechanism of regulation of "purine synthesis" includes differential phosphorylation of proteins including ATase depending on the difference of magnitude and duration of cAMP increase. (1) Am.J.Physiol. 252, G373, 1986, (2) Am.J.Physiol. 253, E684, 1987, (3) Metabolism, 35, 758, 1986

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CONTROLLABLE EXPRESSION OF E. COLI AMIDOPHOSPHORIBOSYLTRANSFERASE (ATase) GENE IN ATase-DEFICIENT MAMMALIAN FIBROBLASTS-A BASIC MODEL FOR GENE THERAPY

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To test the rate-limiting quality of ATase for de novo purine synthesis and the complementarity of a prokaryotic ATase for ATase-deficiency in CHO fibroblasts (CHO-ade) as a basic model for gene therapy, a recombinant between E.coli ATase and a glucocorticoid-responsive MMTV promoter was made. Stable transfectants of CHO-ade with this recombinant, cloned by G-418 cotransfection, were examined for dexamethasone (DEX)-dependent ATase expression at 10^{-6} M. The average ATase activity in 9 transfectants with DEX reached 18.3% of that in a wild type strain of CHOK1, while it was only 4.4% without DEX. Western analysis was performed with anti-E.coli ATase antibody. An E.coli extract showed two bands of 57 and 70K, of which 57K corresponds to a size of purified ATase and a reading frame of 1515bp of cloned DNA. A CHO-ade extract showed no band irrespective of DEX. Extracts of several lines of transfectants showed only one DEX-responsive band of 70K with a good correlation to ATase activity. Based on these data it is suggested that E.coli ATase is bound with other components resulting in a 70K protein both in E.coli and CHO fibroblasts. The complementarity of E.coli ATase for ATase-deficiency of CHO-ade is under way. The recombinant technology affords a valuable means in elucidation of purine synthesis regulation.