53 LOCALIZATION OF XANTHINE DEHYDROGENASE IN LIVER

Y.Hattori, T.Nishino*, C.Usami*, K.Tsushima*, M.Ichikawa**, and A.Ichikawa**.
Dept. of Neurosurgery, Dept. of Biochem.*, Dept. of Anatomy**, Yokohama City Univ. Sch. of Med., Kanazawa-ku, Yokohama 236, Japan

Xanthine dehydrogenase has been reported to be present in an endothelial cell in beef or rat liver and also in peroxisome of a hepatic cell. However, as the most enzyme was recovered in 100,000xg supernatant of rat or chicken liver homogenate, cellular and subcellular localization in liver are still obscure. We reexamined localization of xanthine dehydrogenase in the tissue of chicken liver by immunohistochemical method, or by the method of isolation of hepatic cells. Chicken liver xanthine dehydrogenase purified by the method of Nishino was used for the immunization of rabbit. The antisera obtained from the rabbit gave a distinct single precipitin line in agar against chicken liver xanthine dehydrogenase or crude extract. This antisera were used for immunohistochemical experiments. Chicken liver were perfused with 4% paraformaldehyde, dehydrated with ethanol, embedded in paraffin and sectioned. They were stained with the indirect method using enzyme labeled antibody. The positive staining was observed in the cytoplasm of hepatocytes, Kupffer cells and endothelial cells, suggesting that these type cells contain xanthine dehydrogenase. After isolation of hepatocytes most of xanthine dehydrogenase was recovered in 100,000xg supernatant fraction from homogenate of hepatocytes and the specific activity of enzyme was similar to that in 100,000xg supernatant fraction from homogenate of liver.

54 ADENOSINE DEAMINASE (ADA) EXPRESSED AT HIGH LEVEL IN T LYMPHOCYTES OF A PATIENT WITH ADA DEFICIENCY AND SEVERE COMBINED IMMUNE DEFICIENCY DISEASE (SCID).

Michael S. Hershfield, Joanne Kurtzberg, Sara Chaffee, Emily G. Reisner.
Duke University, Dept of Medicine, Durham, NC, USA

Among patients with ADA deficiency, those with SCID have near complete absence of ADA, while some patients with severe T cell dysfunction but preservation of B cell function may have 1-3% of normal ADA activity in lymphoid cells. Patients with >5% of normal ADA activity in lymphoid cells have had no immune defect. We describe a patient with classical onset of SCID at 3 months, who was begun on red cell transfusions and immunoglobulin replacement therapy at 6 months of age. Despite long term survival, she remained severely lymphopenic and immune deficient. After starting PEG-ADA therapy at age 10 years her lymphocytes increased and became responsive to mitogens, and she developed *in vivo* B cell function. Just prior to starting PEG-ADA, unfractionated nucleated cells from her bone marrow were found to have <1% of normal ADA activity, but IL-2 dependent T cells cultured from this marrow sample had near normal activity (>1000 nmol/h/mg protein). This observation was repeated with blood and marrow derived mononuclear cells and IL2-dependent T cells and T cell clones on 6 other occasions. HLA typing indicated the ADA+ T cells were the patient's own cells. T cell ADA had normal heat stability, Km, pI and was inhibited >99% by EHNA. In contrast to her T cells, 12 EBV transformed B cell clones from the patient had <0.3% of normal ADA activity. ADA+ T cells had normal T cell surface antigens and responsed *in vitro* to IL-2, mitogens, antigen and allogeneic cells. Important questions raised by these findings concern the molecular basis for the T cell ADA activity and the reason that these cells failed to proliferate and function more effectively in this patient *in vivo*. Acquisition of ADA activity by T lymphocyte precursor cells may not be sufficient to restore immune function in some ADA deficient patients. This possibility should be considered in view of proposals to treat ADA deficiency by somatic cell gene replacement.

55 HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE (APRT) DEFICIENCY: A SINGLE MUTANT ALLELE COMMON TO THE JAPANESE.

Yuji Hidaka, Susan A. Tarle, Naoyuki Kamatani, William N. Kelley, and Thomas D. Palella. University of Michigan, Department of Internal Medicine, Ann Arbor, Michigan

Human adenine phosphoribosyltransferase (APRT) deficiency is a relatively common genetic disorder caused by a defective APRT gene, resulting in 2,8-dihydroxyadenine urolithiasis. The kinetic properties of APRT from APRT-deficient Japanese subjects showed similar abnormalities suggesting a distinct "Japanese-type" mutation. Nucleotide sequence analysis of APRT genomic DNA from WR2, a Japanese-type homozygote, identified a T to C substitution in exon 5 on both alleles ($Met^{136}\rightarrow Thr$). RNase mapping analysis revealed that six other Japanese-type homozygotes carried the same mutation on at least one allele. After amplifying the region of the APRT genomic DNA that contains the Japanese-type mutation by the polymerase chain reaction (PCR), dot-blot analysis was performed using normal and mutated allele specific oligonucleotide (ASO) probes. This method demonstrated that all seven Japanese-type homozygotes carried the same mutation on both alleles. The dot-blots clearly distinguish normal, Japanese-type heterozygotes and Japanese-type homozygotes. PCR of genomic sequences and ASO hybridization is a useful tool for detection of common mutations in defined populations, such as "Japanese-type" APRT deficiency.

56 INCORPORATION OF N^4-BEHENOLY-1-β-D-ARABINOFURANOSYLCYTOSINE(BHAC) INTO DNA AS 1-β-D-ARABINOFURANOSYLCYTOSINE(ARA-C)

MASAMUNE HIGASHIGAWA, TOSHIKI OHKUBO, HAJIME KAWASAKI, HITOSHI KAMIYA AND MINORU SAKURAI

Department of Pediatrics, Mie University school of Medicine, Edobashi 2-174, Tsucity, Mieken, 514, Japan

BHAC is a newly synthesized lipophilic derivertive of Ara-C. To clarify its pharmacological mode of action, P388 murine leukemic cells were incubated with two different types of ^{14}C-labeled BHAC, (cytosine-2-^{14}C) BHAC and (acyl-1-^{14}C)BHAC, and DNA was extracted with phenol. And then the phenol extracted DNA was hydrolyzed by nuclease P1 and analyzed with high-performance liquid chromatography(HPLC). The radioactivity of DNA, from the cells incubated with (cytosine -2-^{14}C)BHAC, was detected as Ara-CMP. But the radioactivity of DNA, from the cells incubated with (acyl-1-^{14}C)BHAC, was almost not detected. On the other hand, the main radioactivity of acid soluble fraction was determined as Ara-CTP. On the basis of our results, BHAC is not phosphorylated directly to produce N^4-behenoly-ara-CTP, but mainly once converted to Ara-C and it subsequently produces Ara-CTP, the active metabolite of the drug, and then incorporated into DNA.

57 STUDIES ON THE METABOLISM OF PYRAZINAMIDE AND ALLOPURINOL IN PATIENTS WITH HEREDITARY XANTHINURIA

Kazuya Higashino, Tetsuya Yamamoto, Toshikazu Hada, [1)]Norio Kono, [1)]Masanori Kawachi, [2)]Masahisa Nanahoshi, Sumio Takahashi, Michio Suda and Michiko Naka
The Third Department of Internal Medicine, Hyogo College of Medicine, Hyogo Japan [1)]Osaka University Medical School, Osaka Japan, [2)]Kinki-Central-Hospital of the Mutual Aid Association of Public School Teachers, Hyogo Japan

The aim of this study was to examine whether pyrazinamide and allopurinol were metabolized in three xanthinuric patients of two families of hereditary xanthinuria lacking xanthine oxidase because we were interested in whether both pyrazinamide and allopurinol were oxidized only by xanthine oxidase or by other kinds of oxidase. A xanthinuric patient, the propositus of a family of xanthinuria could neither metabolize pyrazinamide into 5-hydroxypyrazinamide nor allopurinol into oxypurinol. Two xanthinuric patients, the propositus of the other family of xanthinuria and his elder brother could metabolize both pyrazinamide into 5-hydroxypyrazinamide and allopurinol into oxypurinol. These results suggests that xanthinuria consists of at least two subgroups; one does not possess pyrazinamide-allopurinol oxidizing enzyme(s) other than xanthine oxidase or possesses a variant form of xanthine oxidase which can neither metabolize oxypurines nor pyrazinamide or allopurinol, and the other possesses pyrazinamide-allopurinol oxidizing enzyme(s) or possesses the other variant form of xanthine oxidase which can not metabolize oxypurines but can do both pyrazinamide and allopurinol.

58 MOLECULAR BASIS OF INHERITED MYOADENYLATE DEAMINASE (AMP-D) DEFICIENCY.

Peter H. Clarke, William N. Fishbein, Edward W. Holmes, & Richard L. Sabina, Duke Univ. Med. Ctr., Med. Dept., Durham, NC, & Biochem. Dept., Armed Forces Inst. Pathol., Wash, DC, USA.

Inherited AMP-D deficiency is associated with a myopathy characterized by easy fatigability and myalgias. Deficiency of AMP-D is restricted to skeletal muscle and is associated with a decrease in the abundance of immunoreactive muscle-specific peptide. To define the molecular basis for this abnormality we have cloned cDNAs for the skeletal muscle-specific isoforms of human and rat AMP-D. We have made the following observations regarding AMP-D expression in a rodent model and in AMP-D deficient patients: 1) A single gene encodes AMP-D. 2) Two AMP-D transcripts are noted during muscle development in vivo and during myocyte differentiation in vitro. Rat fetal muscle contains an "embryonic" transcript (3.4 Kb) and peptide; post-natal skeletal muscle contains an "adult" transcript (2.5 Kb) and peptide. The latter are restricted to striated muscle. Production of the adult peptide is controlled by transcript switching, increase in transcript abundance during muscle development and possibly efficiency of translation. 3) Four patients with inherited deficiency of AMP-D have been evaluated with these probes. Although immunoreactive peptide is absent from their skeletal muscle, the abundance of the 2.5 Kb muscle-specific transcript is normal. We conclude from these studies that 1) AMP-D expression is controlled at multiple steps during myocyte development 2) Inherited deficiency of the muscle-specific isoform cannot be explained by a defect in transcript switching or rate of transcription, suggesting a defect in translation or a single base substitution.