

5 TWO TYPES OF MOUSE FM3A CELL MUTANTS DEFICIENT IN AMINOIMIDAZOLECARBOXYAMIDE RIBOTIDE TRANSFORMYLASE AND THEIR TRANSFORMANTS ISOLATED BY CHROMOSOME-MEDIATED GENE TRANSFER. Dai Ayusawa, Kiniko Shimizu, Masatake Yamazuchi, Michio Matsuhashi and Takeshi Seno⁴, Saitama Cancer Research Institute, Ina-machi, Saitama-ken 362, Japan, The Institute of Applied Microbiology, University of Tokyo, Tokyo 113, Japan and National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan.

We isolated three adenine auxotrophic mutants (Ade 1-3) of mouse FM3A cells deficient in aminoimidazolecarboxamide ribotide (AICAR) transformylase. Ade 1 and Ade 2 also lacked inosinicase activity. Ade 2 and Ade 3 complemented in cell-cell hybrids, although Ade 1 did not either of them. An intermediate AICAR was found to accumulate in all the mutants indicating that earlier steps than that of formylation of AICAR in the purine biosynthetic pathway seem normal. Primary and secondary transformants were isolated from Ade 2 and Ade 3 using human chromosome-mediated gene transfer. All the transformants tested were found to produce both human-type AICAR transformylase and inosinicase. Unexpectedly, human DNA integrated in the host genome of the certain primary transformant was reduced to as little as several hundred kilobase pairs in length. When digested with certain restriction endonucleases, identical human DNA bands as probed with a human λ u sequence were detected in the transformants of Ade 2 and Ade 3. Since only human chromosome 2 was enough to rescue the genetic defects in the three mutants, these mutants seem to have arisen by a mutation in the AICAR transformylase gene or its adjacent gene.

6 CELL SPECIES-DEPENDENT METABOLISM OF THE POTENT ANTI-HIV (HUMAN IMMUNODEFICIENCY VIRUS) COMPOUNDS 3'-AZIDO-2',3'-DIDEOXYTHYMIDINE AND 2',3'-DIDEOXYCYTIDINE. Jan Balzarini², Erik De Clercq³, Samuel Broder¹ and D.G. Johns¹. Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium and National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

The cytostatic and anti-retrovirus activity, as well as the intracellular metabolism, of 3'-azido-2',3'-dideoxythymidine (AzddThd) and 2',3'-dideoxycytidine (ddCyd) have been evaluated using cell lines derived from different species. AzddThd and ddCyd are considerably more cytostatic to human cell lines than murine cells. In human lymphoid (ATH8, Molt/4F) cells AzddThd accumulates principally as its 5'-monophosphate metabolite (AzddTMP), whereas in murine leukemia L1210 cells it is readily metabolized to AzddTTP. The rapid conversion of AzddThd to AzddTTP in murine cells may explain why AzddThd has a more pronounced activity against Moloney murine sarcoma virus (MSV)-induced transformation of murine C3H cells than human immunodeficiency virus-induced cytopathogenicity in ATH8 cells. In contrast, ddCyd is less active in murine than human cells, most likely due to a differential conversion of ddCyd to its 5'-triphosphate in murine, as compared to human cells. When [³H]-AzddThd and [³H]ddCyd were compared for their metabolism in human (ATH8, Molt/4F) cells, substantial levels of [³H]ddCTP built up gradually, whereas little [³H]AzddTTP was formed even after a 48 hr-incubation period. Thus, much higher ddCTP than AzddTTP levels were achieved in human lymphoid cells, an observation that may be particularly relevant from a therapeutic viewpoint.

7 EXTRACELLULAR NUCLEOTIDE METABOLISM AS THE MAJOR SOURCE OF ADENOSINE IN HUMAN LYMPHOCYTES. Jerzy Barankiewicz, Hans-Michael Dosch, and Amos Cohen, The Hospital for Sick Children, Division of Immunology/Rheumatology, Research Institute, Toronto, Ontario, Canada.

Cells of B lymphocyte lineage (non-E-rosetting B lymphocytes from peripheral blood or tonsils and different B lymphoblastoid cell lines) showed the presence of very active ectonucleotidase sites on their external surface. Thus, B cells are able to metabolize extracellular ATP in extracellular pathway via $AMP \rightarrow ADP \rightarrow AMP \rightarrow$ adenosine. Since adenosine is not produced during intracellular ATP degradation, the degradation of extracellular ATP may be the only source of adenosine production in lymphocytes. The expression of ectoATPase, ectoADPase and ectoAMPase seems to be closely associated with B cell development. EctoATPase and ectoADPase activities increase continuously during B cell maturation (early preB, late preB, mature B cells), whereas ectoAMPase reaches maximal activity in late preB cells. Extracellular nucleotide catabolism seems to have low specificity with comparable degradation rate of dATP, CTP, dGTP and CTP. Extracellular ATP degradation is stimulated by Mg^{2+} but not by Ca^{2+} . These ectoenzymes are not released from cell surface to extracellular space. In contrast to B cells, T lymphocytes (E rosetting lymphocytes from peripheral blood or tonsils) were unable to degrade extracellular nucleotides. These results showed significant difference between extracellular and intracellular ATP catabolism. Intracellular ATP degradation proceeds exclusively via AMP deamination, whereas extracellular via AMP dephosphorylation resulting in adenosine formation.

8 RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR NUCLEOTIDE METABOLISM IN HUMAN LYMPHOCYTES. Jerzy Barankiewicz, Hans-Michael Dosch, Roy Cheung and Amos Cohen, The Hospital for Sick Children, Division of Immunology/Rheumatology, Research Institute, Toronto, Ontario, Canada.

In our previous studies it has been shown that lymphocytes of B lineage but not T lymphocytes are able to degrade extracellular ATP, normally present in human blood plasma (20-30 μM). Adenosine produced by B lymphocytes from extracellular ATP can easily enter the different cells and therefore extracellular nucleotide degradation may significantly affect intracellular metabolism. Adenosine produced extracellularly by cells is mainly deaminated intracellularly to inosine whereas a minor part is incorporated to intracellular nucleotides - mainly ATP. The cells which are unable to degrade extracellular ATP such as T lymphocytes or red blood cells also incorporate adenosine extracellularly produced by B cells to their nucleotides and this incorporation is especially efficient in red blood cells. Although extracellular ATP has no effect on intracellular nucleotide metabolism, its degradation product adenosine significantly inhibits purine biosynthesis *de novo* as well as the salvage of adenine, hypoxanthine, guanosine or thymidine in both B and T lymphocytes. On the other hand, adenosine formed extracellularly may act as a physiological modulator through specific membrane adenosine receptors, and regulate cyclic AMP levels.

9 STUDIES OF MUTANT HUMAN ADENYLOSUCCINATE LYASE. Bruce A. Barshop, Arthur S. Alberts, and Harry E. Gruber, University of California San Diego, Department of Medicine, La Jolla, CA, USA.

Residual adenylosuccinate lyase activity was studied in cultured lymphoblasts from a pair of siblings who have mental retardation and autism, and who have been previously shown to exhibit a deficiency of the enzyme. Utilization of formate by intact cells showed *de novo* synthesis in the mutant lymphoblasts to be similar to normal, consistent with a partial deficiency of this enzyme as previously reported. The steady-state kinetics and thermal stability of adenylosuccinate lyase were examined in the lymphoblast lysates. The pH-activity curves were nearly identical for the mutant and normal cells, with an optimum in the region of pH 7.8. While the substrate affinity was not distinguishable from normal, there was a significant difference ($p < 0.05$) in the V_{max} of the mutant cells. The lyase from normal cells exhibited an apparent K_m of $3.3 \pm 0.8 \mu M$ and a V_{max} of $13.8 \pm 0.9 \text{ nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$. In the mutant cells, the lyase had an apparent K_m of $2.6 \pm 0.5 \mu M$ and V_{max} of $6.7 \pm 1.1 \text{ nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$. There was a significant difference between the normal and mutant cell lysates with regard to thermal stability. At 37°C, the half-time for inactivation was 22.2 hours for the normal cells and 15.7 hours for the mutant cell lysates, and at 60°C the half-times were 5.0 and 0.49 minutes for normal and mutant cell lysates, respectively. There was no evidence of inhibitory activity in the lysates of the mutant cells. The results are consistent with a structural mutation in the adenylosuccinate lyase gene of the affected individuals.

10 CHARACTERIZATION OF A FOLATE-METHOTREXATE TRANSPORT-DEFICIENT CLONE OF LEISHMANIA DONOVANI. Joanne Beck and Buddy Ullman, The Oregon Health Sciences University, Biochemistry Department, Portland, Oregon, U.S.A.

Leishmania donovani is the causative agent of visceral leishmaniasis or kala azar. A clonal mutant strain of *Leishmania donovani* was derived in a single step from a wild type population by virtue of its resistance to 1 millimolar methotrexate. This cell line, MTXA5, was cross-resistant to aminopterin but equally sensitive to two other inhibitors of certain dihydrofolate reductases, pyrimethamine and trimethoprim. In contrast to the wild type parental cells, MTXA5 cells were incapable of taking up or transporting radiolabelled methotrexate and folate from the cell culture medium. Surprisingly, however, both wild type and mutant cells grew equally well in increasing concentrations of folate, although only the wild type parental cells were capable of growing in folate-deficient growth medium supplemented with either bioppterin or neopterin. In order to attempt to analyze the transport system of *Leishmania* biochemically, an affinity labelling technique was developed using radiolabelled methotrexate and folate that had been 'activated' with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Using this protocol, a protein with MW = 46 kd was labelled in wild type cells. This 46 kd protein was associated with plasma membrane fractions. No band was observed in wild type cytosolic fractions. These data have biochemically and genetically identified a common folate/methotrexate carrier in this genus of parasites.