55 ADENOSINE RECEPTORS ON HUMAN T LYMPHOCYTES AND HUMAN THYMOCYTES. Winand N. Dinjens[1], Rineke van Doorn[1], Jan P. van Laarhoven[2], Dirk Roos[1], Wim P. Zeijlemaker[1] and Chris H. de Bruyn[3], [1]Central Lab. Netherl.Red Cross Blood Transf.Service and the Lab of Exp. and Clin. Immunol., Univ. of Amsterdam, [2]Univ. Hosp. Nijmegen, [3]Univ. of Technol., Eindhoven, The Netherlands.

Adenosine (ado) can effect the immune system via binding to cell-surface receptors on lymphoid cells, resulting in an increase in the intracellular level of cAMP. The basal amount of cAMP in T lymphocytes (range 1.6-9.0; mean 5.5 pmoles/10^7 cells) was significantly higher than that in thymocytes (range 0.2-1.7; mean 0.7 pmoles/10^7 cells). Incubation of thymocytes with 10 µM ado resulted in a rapid (1 min), transient increase in cAMP, whereas in T lymphocytes a prolonged (> 15 min) cAMP increase occurred. The effects of ado were most pronounced after incubation (15 min) with the non-metabolizable 2-chloro-ado and the phosphodiesterase inhibitor Ro20-1724. This resulted in a net increase in cAMP levels of 10-35 pmoles for thymocytes and 20-60 pmoles for T lymphocytes. Binding of ado to the ado receptor and the subsequent increase in the intracellular cAMP level may be a major event with respect to the effect of ado on T-lymphoid cells.

56 AMP-DEAMINASE AND CYTOSOLIC 5'NUCLEOTIDASE IN MURINE LYMPHOCYTE SUBPOPULATIONS. Jacques Dornand, Jean C. Bonnafous, Jean Favero, Jean C. Mani. Laboratoire de Biochimie des Membranes, ENSCM, 34075 Montpellier, France.

AMP-deaminase (AMP-DA) which deaminates AMP and dAMP, and cytosolic 5'nucleotidase (c5'N) which hydrolyses intracellular mononucleotides are two enzymes of the nucleotide catabolism which could be involved in the cell ability to prevent cytotoxic accumulation of deoxy-ATP. These enzymes were measured in various mouse lymphocyte subpopulations. AMP-DA levels were found similar in T and B slenocytes and in circulating lymphocytes but were 10-30 fold lower in thymocytes; The low AMP-DA level in thymocytes was explained by the high percentage of immature cortical cells which displayed a 4-6 fold lower activity than the immunocompetent medullary cells. On the opposite the c5'N activities measured in all the mouse lymphocyte subpopulations were identical. These results and the low levels of AMP-DA observed in lymphoblastoid cell lines displaying immaturity caracteristics, showed that the AMP-DA activity varied during lymphocyte differentiation; The observed differences probably arise from the presence of different AMP-DA form in cells at various stages of differentiation, a conclusion suggested by the kinetic data and the specificity of deoxycoformycin (DCF) inhibition of purified AMP-DA preparations from cortical thymocytes and from T splenocytes: The enzyme form present in immature thymocytes poorly deaminated dAMP and was highly inhibited by DCF unlike that present in mature T cells. The AMP-DA activity of the murine lymphocyte subpopulations appeared inversely related to their capacity of dATP accumulation which led in resting lymphocytes to cell death.

57 PURINE AND MONOAMINE METABOLITES IN CEREBRAL SPINAL FLUID. N. Lawrence Edwards, College of Medicine, University of Florida and VAMC, Gainesville, FL, USA, Faye S. Silverstein and Michael V. Johnston, University of Michigan College of Medicine, Ann Arbor, MI, USA.

The end products of purine metabolism in the brain, hypoxanthine (Hx) and xanthine (X), have been used as markers of clinical activity in some disease states. The Lesch-Nyhan syndrome, hypoxic brain damage, and memory loss during depression are all associated with high Hx in the cerebral spinal fluid (CSF). The highly significant correlation between CSF purines (either Hx or X) and CSF monoamines (either HVA or HIAA) in depressed patients (J Neurol, Neurosurg, Psych 46:253, 1983) suggest a direct modulation of monoamine neurotransmitter release by purines. We examined the relationship between the individual CSF concentrations of Hx and X with both HVA and HIAA in 3 Lesch-Nyhan patients (25 CSF samples) and 16 controls (16 samples). No correlation (r=0.2, Pearson) was found between Hx and either HVA or HIAA or between X and either HVA or HIAA in both the Lesch-Nyhan and control subjects. Close correlation (r=0.91) was found between HVA and HIAA in the Lesch-Nyhan patients but not in the controls. Correlation between Hx and X was seen in control spinal fluid (r=0.62) but not in Lesch-Nyhan patients. These findings do not support prior reports of a direct modulatory role for purines in monoamine neurotransmitter release.

58 THE EFFECT OF ALLOPURINOL ON CEREBRAL SPINAL FLUID (CSF) PURINES IN HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE DEFICIENCY SYNDROMES. N. Lawrence Edwards, College of Medicine, University, of Florida and VAMC, Gainesville, FL, USA, Juan G. Puig and Felicitas A. Mateos, La Paz Hospital, Madrid, Spain.

We investigated the effect of allopurinol on CSF purines in 3 Lesch-Nyhan Syndrome (LNS) patients and in one patient with severe partial HGPRT deficiency (Kelley-Seegmiller Syndrome [KSS]). Control spinal fluid hypoxanthine is 7.5 ± 2.0 µM and xanthine is 5.1 ± 2.2 µM. The 3 LNS subjects and the 1 KSS patient had high hypoxanthine levels, 15, 34, 35, and 24 µM, while off allopurinol. Initiation of the allopurinol resulted in a rise in hypoxanthine levels to 58, 50, 50 and 42 µM, respectively. Changes in CSF xanthine and uric acid during allopurinol therapy showed no consistent changes. Xanthine decreased in one LNS patient, slightly increased in another and markedly increased in the third LNS and the KSS patients. CSF uric acid increased in 2 and decreased in 2 while on allopurinol. No allopurinol was detected by high performance liquid chromatography in the CSF but low concentrations of oxypurinol (6-34 µM) were found.

These studies suggest that allopurinol alters CSF oxypurine concentrations in both the "complete" and "partial" HGPRT deficiencies. We also observe that fluctuations in CSF purine levels do not correlate with changes in neurologic behavior in these syndromes.

59 SEPARATE MECHANISMS FOR CELLULAR UPTAKE OF PURINE NUCLEOTIDES BY B-AND T-LYMPHOBLASTS. N. Lawrence Edwards, Annette M. Zaytoun, Gail A. Renard, College of Medicine, University of Florida and VAMC, Gainesville, FL, USA.

Cultured B- and T-lymphocytes have multiple plasma membrane enzymes capable of cleaving extracellular phosphate-containing molecules. These enzymes include a specific 5'-nucleotidase (5'-N, EC 3.1.3.5) whose substrates are primarily purine 5'-nucleoside monophosphates and a non-specific phosphatase (NS-phos, EC 3.1.3.1) which has questionable phosphorolytic activity for purine compounds under physiologic conditions. In a tissue culture system, 2 B-cell and 2 T-cell lines were compared for control growth or killing in the presence of 2 members of the cytotoxic 6-mercaptopurine family:6-MPR (nucleoside) and 6-MPRP (nucleotide). Control growth was comparable for B- and T-cells over 48 hr (330% of initial cell count vs. 280%). 6-MPR and 6-MPRP were equally toxic to B-cells (45% of initial count vs. 55%). The addition of the specific 5'-N inhibitor, AMPCP, completely reversed 6-MPRP induced cytotoxicity but had no effect on 6-MPR toxicity. T-cells are less sensitive to 6-MPRP (110% of initial cell count) than to 6-MPR (65%). AMPCP again had no effect on 6-MPR toxicity and only partially reversed the 6-MPRP toxicity (150% vs. 110%). This persistent T-cell cytotoxicity to 6-MPRP in the presence of AMPCP suggests a different metabolic mechanism for handling extracellular purines when compared to B-cells.

60 HGPRT deficiency with normal PRPP and APRT activity. Bryan T. Emmerson and Ross B. Gordon, University of Queensland Department of Medicine, Princess Alexandra Hospital, Brisbane, Australia 4102

A young man who presented with gout and urate overproduction (24 hour urine urate of 7.7 mmoles/24 hours on a low purine diet) was found to have an HGPRT activity of 9% of normal in an erythrocyte lysate. However the PRPP concentration was consistently normal in such lysates, a finding not previously recorded. In addition, the APRT activity in erythrocyte lysates was also normal, as was the PRPP synthetase activity. Lymphoblasts similarly showed 10% of normal HGPRT activity with a completely normal APRT activity. The incorporation of labelled hypoxanthine or adenine into intact erythrocytes was also normal. The normal APRT activity and the normal incorporation of purine bases into intact erythrocytes are consistent with, and possibly attributable to, the normal PRPP concentration. The question of the mechanism whereby the PRPP concentration can be normal in the presence of HGPRT deficiency will be proposed for discussion.