67 GENETIC EXPRESSION OF ADENOSINE DEAMINASE (ADA) IN HUMAN LYMPHOID MALIGNANCIES. T. Eng Gan, Peter Daddona, and Beverly Mitchell. University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor, MI.

The activity of the purine salvage enzyme, ADA, is 10 to 50-fold higher in T lymphoblasts than in mature lymphocytes and has been used as an enzymatic marker of T-lymphoblastic malignancies. We have asked whether the level of ADA activity in leukemic cells is controlled at the transcriptional or post-transcriptional level. Total cellular RNA was isolated from leukemic cell lines derived from patients with acute T cell leukemia and with T cell leukemia of mature phenotype, as well as from the peripheral blood of 4 patients with non-T cell ALL and 3 patients with chronic lymphocytic leukemia (CLL). Hybridization with a cDNA probe specific for ADA revealed 1.8 and 5.8 Kb bands on Northern blots which were present in all cell types examined. Specific ADA mRNA levels were quantitated by densitometry tracings of Northern blots and compared with both ADA specific activity and ADA protein, as determined by a solid-phase radioimmunoassay. There was a strong correlation between the steady state levels of mRNA and ADA immunoreactive material in all cells examined (correlation coefficient value of 0.76). T lymphoblasts contained 2 to 4-fold more ada mRNA than did the mature T cell lines, 5 to 10-fold more than CLL cells, and 1.5 to 4-fold more than non-T cell ALL cells. ADA specific activity also tended to correlate with mRNA levels. We conclude that the high levels of ADA in T lymphoblasts result from increased transcription of the ADA gene or, possibly, from increased ADA mRNA stability in these cells.

68 EFFECTS OF DIPYRIDAMOLE AND 8-AMINOGUANOSINE ON INOSINE AND GUANOSINE LEVELS IN RAT BLOOD. Richard B. Gilbertsen, Mi K. Dong and Lynn M. Kossarek. Warner-Lambert/Parke-Davis Pharm. Res., Ann Arbor, MI, USA.

The metabolism of inosine (Ino) and guanosine (Guo) was studied in rat blood and plasma in vitro. Nucleoside levels were determined using a modification of the HPLC method of Hartwick and Brown (1977). When heparinized whole blood was spiked with Ino and maintained at room temperature, the level of Ino declined in a linear manner from an initial concentration of 9.9 to 1.6 µg/ml after 60 min., giving a plasma half-life of 23.7 min. Blood spiked with Guo showed a similar linear decline in Guo levels, but in a faster manner (11.0 to 0.1 µg/ml at 60 min., half-life = 8.9 min.). The decline in nucleoside concentration was greatly retarded by maintaining blood and plasma on ice. Addition of dipyridamole (100 nmoles/ml) to whole blood, to block nucleoside uptake, retarded the disappearance of Ino and Guo 1.2 and 2.8-fold, respectively. Addition of 8-aminoguanosine (8-AG, 100 nmoles/ml), an inhibitor of purine nucleoside phosphorylase (PNP), had a slightly greater effect, increasing the half-lives of Ino and Guo 2.5 and 4.4-fold, respectively. Dipyridamole and 8-AG had an additive effect in whole blood. When rat plasma was spiked at room temperature with Ino and Guo (each at 1 µg/ml), neither nucleoside was detectable after 30 min. However, addition of 8-AG to spiked rat plasma totally inhibited the reduction in the levels of each nucleoside for at least 60 min. Thus, membrane transport and especially catabolism by PNP cause disappearance of Ino and Guo from rat blood in vitro.

69 B-LYMPHOCYTES THYMOCYTES AND PLATELETS ACCUMULATE HIGH dATP LEVELS IN SIMULATED ADA DEFICIENCY

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Novel findings in vitro and in vivo obtained previously in simulated and inherited ADA deficiency were investigated using [8-^{14}C] deoxyadenosine (dAR) in short-term experiments in intact human cells of the myeloid and lymphoid series.

The studies produced several interesting results. (1) Tonsil-derived B-lymphocytes, thymocytes and platelets all accumulated detectable amounts of dATP even without ADA inhibition, and together with erythrocytes, extremely high dATP levels when ADA was inhibited by deoxycoformycin (dCF): varying amounts of dCF (20-60µm) were needed to completely inhibit ADA depending on the cell type. (2) By contrast, dATP accumulation by peripheral blood lymphocytes, granulocytes and macrophages was negligible without, and extremely low even with dCF. (3) B-lymphocytes showed a capacity equal to that of thymocytes in their ability to sustain the elevated dATP levels accumulated in ADA deficiency conditions

The results support earlier findings which question the hypothesis that B-cells, compared with T-cells, have an inherent resistance to the toxic effects of dAR because of a lower ability to accumulate and sustain elevated dATP levels. They underline the difficulty in extrapolating from lysed or cultured cells to the situation in vivo in the peripheral blood. They suggest that the severe combined immunodeficiency in this disorder may be due to an equal sensitivity of B-lymphocytes and T-lymphocyte precursors to the toxic effects of dATP accumulation.

70 ENHANCEMENT OF T CELL PROLIFERATION AND DIFFERENTIATION BY 8-MERCAPTOGUANOSINE. Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, Department of Immunology, La Jolla, Calif., U.S.A.

Studies were undertaken to investigate the ability of the C8-substituted guanine ribonucleosides to modulate the proliferative and/or differentiative activity of T cells. This family of substituted nucleosides has been found to induce B cells to undergo proliferation and differentiation. However, T cells and thymocytes alike do not proliferate in response to 8MGuo. Moreover, this nucleoside does not modulate T cell proliferation evoked by either the mitogenic lectin Con A or by IL-2. 8MGuo can, however, modulate the T cell proliferative response to allogeneic cells. Because of earlier, vigorous B cell proliferation elicited by the nucleoside, modulation of T cell allogeneic responses must be observed in the absence of B cell responses. This can be done by stimulating thymocytes with irradiated cells and 8MGuo in the presence of supplemental IL-2, or by stimulating SJL spleen cells (whose B cells are hyporesponsive to 8MGuo) with irradiated allogeneic cells in the presence of 8MGuo. An analogous situation pertains to T cell function in that only certain functions can be modulated by 8MGuo. That is, T cells can not generate T cell growth or B cell differentiation activity in the presence of 8MGuo alone. Moreover, this nucleoside does not induce polyclonal cytotoxicity in T cell populations. It does, however, enhance generation of H-2-restricted CTL induced by allogeneic cells. Thus, in contrast to the situation pertaining to B cells, 8MGuo acts as an adjunct, but not as an initiator for T cell proliferation and differentiation.

71 ANTIGEN-SPECIFIC ENHANCEMENT OF THE HUMAN ANTIBODY RESPONSE BY A SUBSTITUTED NUCLEOSIDE. Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, Department of Immunology, La Jolla, CA, USA

The antigen-specific primary antibody response of human lymphocytes in vitro was studied with respect to dependency upon interleukin 2 (IL-2) and subsequent modulation by C8-substituted guanine ribonucleosides. The specific response to sheep erythrocytes was shown to be dependent upon IL-2. Addition of optimal concentrations of the nucleoside, 7-methyl-8-oxoguanosine (7m8oGuo), to cultures containing antigen and IL-2, caused marked amplification of the underlying antibody response. Synergy between 7m8oGuo and IL-2 was antigen-dependent and could not be accounted for by the independent antigen-specific and nonspecific components. That IL-2 itself was responsible for both the specific response to antigen and synergy with 7m8oGuo was confirmed by use of recombinant IL-2. 7m8oGuo enhanced the response to antigen in a dose-dependent fashion. Kinetic studies demonstrated that this nucleoside acts in the context of an ongoing immune response, because its addition could be delayed up to 3 days without loss of activity. The ability of 7m8oGuo to bypass the requirement for intact T cells in this response was substantiated by investigating the ability of B cell-enriched populations to respond to the T-dependent antigen, SRBC, in the presence and absence of 7m8oGuo. T cell-depleted populations were capable of responding to antigen in the presence of 7m8oGuo so long as IL-2 was also present. These data demonstrate that a simple nucleoside analog can amplify the human antibody response in an antigen-specific manner and may act as an alternate source of T cell help.

72 INHIBITION OF DE NOVO PURINE SYNTHESIS BY METHYLTHIOADENOSINE. Ross B. Gordon and Bryan T. Emmerson, University of Queensland Department of Medicine, Princess Alexandra Hospital, Brisbane. 4102. Australia.

De novo purine synthesis in human B lymphoblast cell lines was inhibited by methylthioadenosine (MTA) or adenine in the medium. Two human leukemic T-cell lines lacking methylthioadenosine phosphorylase (MTAP) activity showed increased rates of de novo purine synthesis which were resistant to inhibition by MTA but were inhibited by adenine in a similar fashion to MTAP$^+$ cells. The presence of MTA in culture media inhibited growth in a dose-dependent manner in MTAP$^+$ and MTAP$^-$ cell lines. Similar inhibition of growth was effected by the presence of adenine in the medium. These results suggest that the inhibition of de novo purine synthesis by MTA is due to cleavage of the MTA to adenine by the MTAP enzyme. The adenine so formed can be converted to 5'-AMP in a PRPP-dependent reaction catalysed by the purine salvage enzyme APRT.

Inhibition of growth of the MTAP$^-$ cell lines by MTA suggests that MTA may also exert effects on cell metabolism by a mechanism unrelated to its degradation.