62

Altered Expression Of Inhibitory Cytokines, Interleukin-4 (IL-4) and Transforming Growth Factor-Beta1 (TGFβ1) In Bone Marrow Stromal Cells Induced By The Ecotropic Murine Leukemia Virus (MuLV)

Oliver R. Oakley[a,b], Nedda K. Hughes[b], Vincent S. Gallicchio[b,*] and Nicholas J. Birch[a]

[a] *Biomedical Research Laboratory, School of Health Sciences, University of Wolverhampton, Wolverhampton, WV 1DJ, UK*
[b] *Division of Hematology/Oncology, Departments of Internal Medicine, Clinical Sciences, Microbiology and Immunology, and Toxicology, Lucille P. Markey Cancer Center, Chandler Medical Center, University of Kentucky, Lexington, KY, 40536-0084, USA*

Murine acquired immunodeficiency syndrome (MAIDS) can be induced in susceptible mouse strains by a unique mixture of murine leukemia viruses (MuLV), designated LP-BM5. This mixture consists of three viruses, a replication competent ecotropic (PBM5-eco), a mink cell focus forming (MCF) and a replication defective virus. Although the pathogenesis of the disease still remains unclear, infection can be characterized by a shifting pattern in cytokine expression. In this study stromal cell lines (KTLT2) were infected with PBM5-eco alone or the LP-BM5 mixture. At various time points after infection, both the expression of the LP-BM5 and PBM5-eco, and expression of the inhibitory cytokine genes were assessed. LP-BM5 infection showed increased message for TGFβ1 compared to that of normal non-infected controls. Moreover, when cultures were infected with PBM5-eco, the expression of TGFβ1 was also increased, although not as significantly as with LP-BM5. These data suggest that the ecotropic MuLV has a more complex role than simply a replication 'helper' in the spread of the defective MuLV, as previously thought. It may act in a synergistic effect with other components of LP-BM5 to contribute to the cytokine dysregulation which is associated with the progression of MAIDS.

63

Zidovudine Induces Alterations of Apoptosis and Resistance to Antineoplastic Agents in T-Cell Lymphoma Cells

R. Kotchetkov[1,2], B. Gröschel[1], J. Cinatl[1,2], B. Kornhuber[2], H. Rabenau[1], J. Cinatl, Jr.[1]
[1]*Institute of Med. Virology,* [2]*Center of Pediatrics, Department of Hematology and Oncology, J.W. Goethe-University, Frankfurt M, Germany*

Prolonged treatment of leukemic cell lines with antiretroviral agents such as nucleoside analogues may result in the development of cell resistance against antiviral agents. We tested whether prolonged treatment of H9 cells (T-cell lymphoma cell line) with 3'-azido-3'-deoxythymidine (AZT) interferes with the sensitivity to antitumor agents commonly used for treatment of AIDS-associated lymphomas. H9 cells grown for more than two years in medium containing 250 μM of AZT were at least 100-fold less sensitive to cytotoxic effects of AZT than parental H9 cell line. These cells designated H9^{r}AZT^{250} were 5- to 20-fold less sensitive to toxic effects of antitumor agents including cisplatin (CP), vincristine (VIN), doxorubicin (DOX) and etoposide (VP-16). The resistance to antitumor agents was associated with inhibition of apoptosis as demonstrated by the terminal deoxynucleotidyl transferase-mediated nicked-end labeling (TUNEL) assay and DNA fragmentation assay. The regulation of genes involved in the regulation of apoptosis such as *bcl-2* was changed in H9^{r}AZT^{250} cells. The results demonstrate that prolonged treatment of tumor cells with AZT may result in the development of resistance to antineoplastic agents due to the alterations in apoptosis.

64

HIV-1 Tat Protein Potentiates Zidovudine-induced Bone Marrow Suppression. Krishna C. Agrawal, S. Chitnis and D. Mondal, Dept. of Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112.

The *trans*-activator protein (Tat) of HIV-1 plays an important role in viral pathogenesis. We have earlier reported that the viral Tat protein inhibited butyric acid-induced hemoglobin production in K562 cells, a human hematopoietic progenitor cell line. We have further suggested that Tat may play a significant role in abrogating hematopoietic function in AIDS patients. We now report that Tat can significantly enhance AZT-induced bone marrow suppression. Differentiation of K562 cells via either erythroid (induced by butyric acid, 1.4 mM) or megakaryocytic (induced by phorbol ester, 10 nM) lineages, were suppressed by Tat protein as monitored by benzidine positivity and ^{3}H-serotonin uptake, respectively. AZT, at 5 μM, inhibited hemoglobin (Hb) production in K562-*tat* cells by 55 % and at 20 μM, by 80 %. AZT also down-regulated the γ-globin gene expression at these concentrations by 30 and 80%, respectively. Expression of erythropoietin receptor mRNA levels was inhibited by 90% at 5 μM AZT in the presence of Tat in these cells whereas in the absence of Tat the inhibition was only 35%. We have further shown that there is a constitutively higher production (2-fold) of TGF-β in K562-*tat* cells indicating that the release of this cytokine may have an important role in inhibiting differentiation of the progenitor cells. In our previously published studies we have shown that overexpression of poly-TAR RNAs in K562 cells can counteract the suppressive effect of Tat on hematopoietic differentiation. Thus, Tat-specific inhibitors in addition to cytokine therapy may have a role in overcoming AZT-induced hematopoietic toxicity in AIDS patients. (Supported by the NIH grant AI 32893).

65

Incorporation of Anti-HIV Nucleotide Analogs and Nucleoside Phosphonates into DNA by Human DNA Polymerases α, β and γ. T. Cihlar and M. S. Chen. Gilead Sciences, Foster City, CA 94404, USA.

Incorporation of selected diphosphates of nucleoside phosphonates and triphosphates of currently approved anti-HIV nucleoside analogs into DNA by human DNA polymerases α, β and γ has been studied. All three polymerases were able to incorporate diphosphates of 9-(2-Phosphonomethoxy-ethyl)adenine (PMEApp), 9-(2-phosphonomethoxyethyl)guanine (PMEGpp), (R)-9-(2-phosphonomethoxypropyl)adenine (PMPApp), (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine (PMPDAPpp) and (2R,5R)-9-[2,5-dihydro-5-(phosphonomethoxy)-2-furanyl]adenine (D4APpp) into primer/template DNA of defined sequence. After incorporation, these nucleoside phosphonates act as terminators of primer extension. Kinetic constants of their incorporation were determined and compared with those for incorporation of triphosphates of 2',3'-dideoxyadenosine (ddATP), 2',3'-dideoxycytosine (ddCTP), (-)-2'-deoxy-3'-thiacytidine (3TCTP), 2',3'-didehydro-2',3'-dideoxythymidine (D4TTP) and 3'-azido-3'-deoxythymidine (AZTTP). Relative efficiencies of incorporation (i.e. percentage of incorporation efficiency of corresponding natural deoxynucleoside triphosphate) by DNA polymerase α range from 0.05% for 3TCTP to 51% for PMEGpp. DNA polymerase β catalyzes the incorporation with relative efficiencies ranging from 0.014% for AZTTP to 125% for ddCTP, and efficiencies of incorporation by DNA polymerase γ vary between 0.13% for 3TCTP and 25% for ddCTP. Generally, the lowest incorporation efficiencies with all three polymerases have been found for PMPApp (0.06 - 1.4%) and PMPDAPpp (0.075% - 2.2%). Data presented in this study demonstrate remarkable differences in the incorporation efficiencies of various antiviral nucleotides by three host DNA polymerases and may, in part, explain some aspects of their cytotoxicity.