41A

APOPTOTIC CELL DEATH DURING TREATMENT OF HUMAN LEUKEMIA. F. Traganos*, X. Li, E. Feldman, K. Seiter, and Z. Darzynkiewicz, Cancer Research Inst. and Dept. of Med., New York Med. College, Valhalla, NY.

Apoptosis-associated DNA strand breaks were detected *in situ*, in individual bone marrow or peripheral blood cells of patients with leukemia (ALL, AML, CML and APL), prior to and during chemotherapy. The DNA strand breaks were labeled with digoxigenin- or biotin- conjugated dUTP in a reaction catalyzed by terminal deoxynucleotidyl transferase. Incorporation of dUTP was quantitated using fluorescein labeled avidin or antidigoxigenin. The cells were counterstained for DNA with propidium iodide and analyzed by bivariate flow cytometry. DNA strand breaks in pretreatment samples varied from 0.1% to 16%, with a majority exhibiting less than 3% apoptotic cells. Administration of DNA topoisomerase I (topotecan) and II (mitoxantrone, VP-16) inhibitors, antimetabolites (ara-C) or microtubule poisons (taxol) triggered the appearance of cells with extensive DNA strand breaks. Peak response (percentage increase of pretreatment values) occured from 8-24 h or 48-72 h following initial administration of topoisomerase inhibitors or taxol and ara-C, respectively. The prognostic value of the apoptotic index (before and during treatment) and the kinetics of apoptosis induction are being investigated to determine their ability to predict response to therapy and duration of remission.

42B

COMPARATIVE STUDY OF FLOW CYTOMETRY AND IMAGE ANALYSIS IN SITU APOPTOSIS DETECTION OF CELLS INDUCED BY HEAT SHOCK AND DEXAMETHASONE. O.J. Trask [*1], G.N. Bijur [2], F.M. Robertson [2], The Ohio State University Comprehensive Cancer Center, Analytical Cytometry Laboratory, Columbus, Ohio[1] and The Ohio State University Department of Medical Microbiology and Immunology, Columbus, Ohio[2].

Quantifying detectable apoptotic bodies in the same sample by flow cytometry and image analysis allows both the analyses of large cell numbers for statistical purposes and the visual identification of single cell morphological characteristics. Jurkat cells, mouse thymocytes, and human peripheral blood lymphocytes were triggered with a specific stimulus that induced apoptosis within 3 to 6 hours. Following stimulation, terminal deoxynucleotidyl transferase and anti-digoxigenin antibody were added to fixed cells. Programmed cell death was identified by end labeling of the 3'-OH DNA fragments in cells responding to stimulation with 1uM dexamethasone for 4 hours at 37°C, and to cells exposed at 42°C (heat shock) for 1 hour. Propidium iodide stained cells examined by flow cytometry showed an increase in apoptosis (sub G0/G1) with a decrease in S phase and G2/M. Visual examination by image analysis revealed a decrease in cell size. The detection of apoptotic cell populations by a combination of flow cytometry and image analysis provides a sensitive means for an accurate determination of the rate and extent of cell proliferation and cell death in a variety of biological systems using cultured cells and tissue derived cells.

43C

EARLY FUNCTIONAL CHANGES IN HUMAN T-LYMPHOCYTES INDUCED TO APOPTOSIS BY X-IRRADIATION OR CORTISONE

T.Elsherif, H.Kahle, S.Klingel, S.Ganesh, G.Valet*

Arb.Gruppe Zellbiochemie, Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany

Human Jurkat T-cells were X-irradiated (20Gy, 280KV, 2mm Cu-filter, 2Gy/min) or cultured in the presence of 1.7µM dexamethasone-21-acetate. Both apoptosis inducing treatments caused several **early functional changes** in viable cells such as **increased oxidative activity** (2,7-dichloro-fluorescin-diacetate, DCFH-DA), **transmembrane** (DiOC6(3)) and **mitochondrial** membrane potentials (rhodamine123), intracellular **Ca^{2+}** (INDO1) within 15 to 30min followed by **glutathione** (ortho-phthaldialdehyde, OPT) increase and elevated **DNA stainability** (acridine orange, AO, 8µg/ml) after 30 to 60min. Oxidation sensitive **cysteine proteinase** cathepsin B,H,L (rhodamine110 substrate $(Z\text{-}Arg)_2$R110) but additionally cathepsin G **serine** proteinases $(Z\text{-}Ala)_2$R110 and **glutathione** levels were **decreased** while aminopeptidase **metalloproteinases** (Leu_2-R110, Phe_2-R110) as well as intracellular **pH** values (1,4-diacetocy-2,3-dicyanobenzene, ADB) transiently **increased** between 60-120min. All changes occured well before DNA ladder formation on gels after 3h or numerical cell decrease after 6h, indicating that substantial intracellular processes precede final DNA degradation. Besides other interpretations, the results are compatible with the view that apoptosis induction may be due to a **temporarily imbalanced oxidative stress.**

44D

ADVANTAGES OF APOPTOSIS ANALYSIS USING THE ATTRACTORS™ PROGRAM. E. VanBuren*, M. Cankovic, and S. Lerman, Dept. of Imm. and Micro., Wayne State Univ., Detroit, MI 48201.

Apoptosis data from three experiments were analyzed using two strategies. In each experiment lymph node cells from tumor-bearing (RCS5) cyclophosphamide-treated SJL/J mice were examined for percent apoptosis of CD4+ cells after in vitro culture with irradiated tumor cells (stimulated), or after culture in medium alone (unstimulated). Listmode data were collected on a FACS Vantage using LYSYS II. Network software was used to transfer data to a PC and a Macintosh. The first strategy entailed a two stage method. Stage one involved using PC-LYSYS to gate on CD4+ cells, excluding debris and doublets. In stage two, saved histograms were analyzed using ModFit (modification of F_DIP_N2.MOD) to determine percent apoptosis in the CD4+ population. The second strategy entailed defining an attractor set that determined the percent of apoptotic CD4+ cells, excluding debris and doublets. Linear regression showed that both strategies were comparable (r=0.999 stimulated, r=0.999 unstimulated). The advantages of the Attractors strategy were: 1) the ability to automate analysis through the adaptability of attractors to slight population shifts; 2) the convenience of saving the complete analysis method in an attractor set for subsequent use; and 3) the ease of outputting results to a spreadsheet. Supported by CA52603.

45A

COMPARISON OF CELLULAR AND NUCLEAR METHODS FOR DISCRIMINATING APOPTOTIC CELLS BY FLOW CYTOMETRY. R M Zucker[1*], K H Elstein[1], and J M Rogers[2]. [1]ManTech Env. Tech, Inc./[2]USEPA, RTP, NC.

We compared cellular flow cytometric techniques employing carboxyfluorescein (CF), Höechst 33342, and Höechst 33258 with a nuclear technique in their ability to discriminate apoptotic subpopulations in rat thymocyte cultures exposed to dexamethasone or tributyltin (TBT) for 1 or 4 hr. The nuclear technique, which detects apoptotic cells as a single low-fluorescence peak, underestimated the percentage of affected cells and did not distinguish early and late apoptotic cells. In the cellular techniques, early apoptotic cells exhibited increased fluorescence, while late apoptotic cells exhibited either maximal or minimal fluorescence, depending on the fluorochrome used. With respect to sensitivity to staining conditions, Höechst 33258 was the most stable, Höechst 33342 the least, and CF fluorescence varied not only with time but with TBT concentration, as a consequence of decreased membrane fluidity. These results suggest that, of the methods investigated, the method of choice depends on the goal of the analysis, with Höechst 33258 being the most stable for discriminating the largest number of subpopulations.

(This abstract of a proposed presentation does not necessarily reflect EPA policy.)