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MODULATION OF RHODAMINE-123 EFFLUX BY CYCLOSPORIN A IN ACUTE LEUKEMIA: PROGNOSTIC IMPLICATIONS. A. Tafuri, MT. Petrucci, L. Burba*, MR. Ricciardi*, R. Stocchi*, MG. Mascolo*, C. Guglielmi*, G. Avvisati, L. Annino*, AM. Testi*, F. Mandelli. Hematology, Department of Human Biopathology, University "La Sapienza", Rome, ITALY

Expression of the multidrug resistance (MDR) gene, as detected by MDR1 mRNA and its product P-gp 170, is reported to have prognostic importance in cancer. We investigated the MDR expression in Acute Myeloid (AML) and Lymphoid Leukemia (ALL) patients (pts) by a functional flow cytometric assay based on the fluorescent dye Rhodamine-123 (Rhd) Efflux (Rhd-E), performed in the presence or absence of Cyclosporin A (CsA), used as MDR-reversing agent. Within each sample the percent decrease of mean fluorescence with/without CsA was compared. Rhd-E was not seen in the CEM parental cell line whereas in the derived CEM/VBL100 resistant cells it was markedly present and blocked by CsA (71.3%). The functional test was correlated with P-gp 170 expression and MDR1 mRNA, detected by flow cytometric measure of 4E3 MoAb and RT-PCR respectively. We studied 98 pts with acute leukemia, 54 at diagnosis and 44 in relapse, 52 with AML and 46 by ALL. A significant mean (m) lower ($p< .000$) Rhd-E was found in 12 acute promyelocytic leukemias (APL) (m=1%) compared to the M1 and M2 (n=15) FAB subtypes, as well as in 7 pediatric AML (m=3.1%) compared to 30 adult pts ($p=.05$). AML at diagnosis showed a lower (m=13.6%) Rhd-E compared to pts in relapse (m=25.6%). Among the 44 evaluable pts, 27% failed to achieved complete response (CR). They showed higher Rhd-E in the overall population (m=23.9%) as well as in the pts at diagnosis (m=21.2%) and in relapse (m=29%). A significant difference ($p=.04$) was found among the AML cases younger than 65 years when responding pts were compared with those that failed to achieve CR (m=9.7% vs 23.5%). Rhd-E higher than 5% was found in 52.2% of the pts and this value was detected in 77% of AML at diagnosis that did not achieve CR whereas a Rhd-E < 5% was found in 70% of the responding pts ($p=.03$). Evaluation of ALL confirmed also in these pts a significantly ($p=.02$) higher Rhd-E between pts studied in relapse and at diagnosis (m=15.3% vs 7.3%). Only a minor difference was found in ALL between pts that failed to achieve CR and those who responded (m=15.5% vs 10.1%). In ALL pts 65.2% of them showed a Rhd-E > 5%, this was found in 66.6% of pts at diagnosis that did not achieve CR, whereas in 50% of responding pts Rhd-E was < 5%. Among 26 pts tested for 4E3, Pgp-170 was found in 53% of AML and 45% of ALL cases. MDR measurement by 4E3 appeared less sensitive than the functional Rhd-E test. Preliminar data on RT-PCR showed expression of MDR1 in 32% and 28% of AML and ALL pts tested respectively. In conclusion: 1)Adult AML pts unable to achieve CR showed an high Rhd-E, 2)Pediatric AML and APL are characterized by lower Rhd-E, 3)Higher Rhd-E was found in relapsed ALL and AML, compared to pts at diagnosis 4)No correlation was found in ALL between functional test and achievement of CR

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CLINICAL RELEVANCE OF P-GLYCOPROTEIN (PGP) EXPRESSION DETERMINED BY FLOW CYTOMETRY IN ACUTE MYELOID LEUKEMIA (AML). G.Del Poeta*, A.Venditti*, G.Aronica*, R.Stasi*, M.Masi*, A.Bruno*, M.C. Cox*, F.Pisani*, M.D.Simone*, M.Tribalto*, R.Iazzoni* F.Buccisano*, G.Papa. Cattedra e Divisione di Ematologia, Universita' "Tor Vergata", Ospedale S.Eugenio, Roma, Italy.

Overexpression of PGP has been identified as an underlying mechanism of multidrug resistance (MDR) and appears to be closely related to clinical resistance in newly diagnosed and relapsed AML patients. We studied 146 patients with *de novo* AML, 69 females and 77 males, median age 56 yrs (range 17-81).They were treated by intensive chemotherapy including an anthracycline , etoposide, and high doses of cytosine arabinoside. PGP expression was analyzed using either the fluorescein isothyocyanate (FITC) conjugated C219 MoAb (CIS Diagnostici, Vercelli , Italy) in 146 cases or unconjugated JSB1 MoAb (Seralab, Sussex, UK) in 97 patients. As these MoAbs recognize an epitope on the inner surface of the cytoplasmic membrane, the blast cells were permeabilized in 3.5% paraphormaldeyde/PBS and, after two washings in PBS, in 50% cold acetone/PBS. Analysis was carried out by flow cytometry (Epics Profile, Coulter, FL, USA). Given the heterogeneous expression of PGP in terms of number of cells stained or fluorescence intensity, the threshold of positivity was set to a conventional 20%. In leukemic blasts, the differences in fluorescence intensity between control sample and test sample were often small, resulting in nondisjunct distributions. Therefore, an improved histogram analysis (IMMUNO-4, Coulter, FL, USA), based either on cumulative subtraction or normalization of the control histogram to best match the test histogram, was applied to distinguish antigen-positive from antigen-negative cells. Moreover, we used rhodamine 123 (Rho) staining and flow-cytometry analysis in 40 cases as a simple functional test of MDR1 activity and correlated the results with P-170 phenotype and clinical outcome. Sixty-six samples (45.2%) were considered positive for P-170 expression detected by C219 and sixty-five (67%) positive using JSB1. There was a strict correlation among C219 and JSB1 positivity: all the C219+ cases were positive for JSB1 MoAb ($P < 0.0001$). High leukocyte count ($>50 \times 10^9$/L) was related only to JSB1 positivity ($P= 0.02$). Significant correlation was found between the surface marker CD7 and both C219 and JSB1 ($P< 0.001$ and < 0.01, respectively). All the patients positive for C219 and/or JSB1 showed significant decreased mean Rho fluorescence compared to PGP negative ones ($P < 0.01$). With regard to clinical responsiveness, PGP+ AML patients, either C219+ or JSB1+, showed lower complete remission (CR) rates ($P< 0.001$) and shorter overall survival ($P= 0.001$ and $P= 0.002$, respectively) compared to the PGP negative ones. Our study contributes to the identification of patients with poor prognosis, but also suggests that therapeutic trials with P-170 blockers may be important, mainly for patients with MDR-1 positive blast cells.

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RAS MUTATIONS IN CHINESE PATIENTS WITH BENZENE-ASSOCIATED AND *DE NOVO* ACUTE LEUKEMIA. N.E. Mills, R.B. Hayes*, C.L. Fishman*, S-N. Yin*, M.L. Linet*, L.B. Travis*, and D.R. Jacobson*. Department of Medicine, New York University Medical Center, New York, NY, Epidemiology & Biostatistics Program, National Cancer Institute, Bethesda, MD, and Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, China.

Activating point mutations in the N-*ras* oncogene have been reported to occur in 22% of cases of acute myelocytic leukemia (AML) and in 15% of cases of acute lymphocytic leukemia (ALL). Benzene exposure increases the risk of acute leukemia, and occupational exposure to chemicals has been linked to *ras*-positive AML. To study the association of benzene exposure with patterns of DNA damage, we determined the frequency and types of N-*ras* mutations in patients with acute leukemia, with and without benzene exposure. Stained peripheral blood or bone marrow aspirate slides were obtained from patients from China with acute leukemia (13 AML, 3 ALL) including 8 benzene-exposed factory workers and 8 with no known chemical exposure history. DNA was extracted from the material scraped off these slides, and analyzed for point mutations in N-*ras* codons 12, 13, and 61 by a sensitive assay able to detect 0.1% mutant alleles. N-*ras* exons 1 and 2 were PCR-amplified using mismatched primers which introduced new restriction sites into PCR products derived from normal, but not mutant alleles. Restriction enzyme digestion and further PCR were performed to enrich for mutant alleles, with mutations indicated by a digestion-resistant PCR fragment on ethidium bromide-stained agarose gel electrophoresis. Mutation-containing samples were further amplified using different mismatched primers to introduce new restriction sites, dependent upon the mutation present, into the PCR products; these products were then digested with the appropriate enzyme to identify the mutation present. An N-*ras* codon 12 mutation was found in 5/8 benzene-exposed patients (4 AML, 1 ALL) and 5/8 patients without known chemical exposure (3 AML, 2 ALL). Overall, mutations were found in 7/13 cases with AML (54%), including 3/3 M3 cases, and in all 3 ALL cases. All 10 mutations were G to A transitions at positions 1 or 2 of codon 12 (GAT, n=7; AGT, n=3). The predominance of G to A transitions is consistent with previous reports in *ras*-positive AML. No mutation was found in N-*ras* codon 13 or 61. Thus, the prevalence and type of N-*ras* mutations was not different for patients with and without benzene exposure. The prevalence of *ras* mutations in AML (54%) is higher than the 22% reported in the literature ($p=.014$, 2-tailed t-test). The most likely explanation for the increased prevalence of mutations in this study is that our assay is 2 orders of magnitude more sensitive than commonly-used assays for *ras* mutations. Alternatively, Chinese patients with acute leukemia may have a higher prevalence of *ras* mutations than other groups. We conclude that the frequency and type of *ras* mutations are not different for benzene-exposed and *de novo* acute leukemia, and the prevalence of N-*ras* mutations in these patients is higher than has been reported in studies using less sensitive assays of predominantly non-Asian populations.

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THE PATHOGENESIS OF INTERLEUKIN-2 CYTOTOXICITY TO HUMAN STEM CELL LEUKEMIA IN SCID MICE. A.M. Dunk,* C.A. Clark,* D. Lombardi,* J. Kirsch,* K. Weinberg, J. Kurtzberg. Duke University Medical Center, Durham, NC, LA Children's Hospital, Los Angeles, CA, and the National Cancer Institute, Bethesda, MD.

Human Stem Cell Leukemia (hSCL) is a leukemic syndrome characterized by proliferation of CD7+, lineage negative (CD7+LN-) cells. Previous studies suggest that CD7+LN-cells represent an early T cell precursor retaining the capacity for multilineage differentiation. Our laboratory has established human CD7+LN-leukemic cells as a serial transplantable model of this leukemia in SCID mice called hSCL. Over 35 months, hSCL has been serially passaged through 33 cohorts of mice where it reproducibly disseminates, causing fatal leukemia in approximately 30 days. Early treatment with 3 doses of intraperitoneal polyethelene-glycol interleukin-2 (PEG-IL2) significantly delays the onset of clinical leukemia and death. Our current focus of investigation is to determine the mechanism of action by which IL-2 exerts its antileukemic effect. The following three hypotheses have been tested and rejected: **(1) IL-2 activates murine immune cells against hSCL:** Treatment of mice with anti-asialo GM1 antibody, total body irradiation, murine IL-2, and adoptive transfer of splenocytes and plasma from IL-2 treated mice failed to show evidence of this mechanism; *in vitro* assays for LAK, NK, and CTL activity also failed to support this hypothesis. **(2) IL-2 activates normal human immune cells transferred to the mice along with the injected leukemia:** Evidence against this hypothesis includes a lack of human T-cells detectable by PCR for the TCRcβ2 gene and a karyotype failing to demonstrate any cells with a normal human karyotype in hSCL-bearing mice or hSCL cultured *in vitro* with IL-2. **(3) IL-2 induces differentiation of hSCL:** Phenotypic analysis of hSCL from IL-2 treated mice did not show an increase in expression of mature T-cell antigens. The sum of these results has led us to our current hypothesis: **(4) IL-2 is directly cytotoxic to hSCL:** *in vitro* studies of hSCL cultured with IL-2 support this hypothesis. Following 6-7 weeks in culture, significantly retarded growth rates compared with controls and evidence of apoptosis (by propridium iodide staining of DNA and by electron microscopy) are present in the IL-2 cultures. This effect may be mediated by a known or novel IL-2 receptor (IL2R). Low levels (1-11%) of surface IL2Rα and IL2Rβ subunits are expressed on hSCL cells, and mRNA for each of the α, β, and γ receptor subunits is detectable in both unstimulated and in IL-2 treated cells. The significance of the expression of these receptor subunits is not yet known. Current work is focused on the signal transduction pathways used by IL-2 in its interaction with hSCL. In *in vivo* studies, Rapamycin and Cyclosporine A, drugs which block the S6 kinase signaling pathway of IL-2, have failed to block the antitumor effect of IL-2 on hSCL in SCID mice. Studies in progress are searching for expression and phosphorylation of tyrosine kinases thought to be involved in IL-2 signaling in treated and untreated hSCL.

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