5

PROVIRAL SEQUENCES OF HTLV-I IN PATIENTS WITH CTCL

Böni R, Burg G, Salvekar Anu*, Crooks Carol*, Fuchs D, von der Helm K** and Wood G*
Dep. of Dermatology, Zürich, Switzerland. *Case Western Reserve University, Cleveland, Ohio, USA. **Pettenkofer-Institute, Munich, Germany.

Recently, there has been much interest concerning the hypothesis of viral pathogenesis of cutaneous T-cell-lymphoma. For the adult T-cell lymphoma the viral etiology of HTLV-I has been determined. Using conventional screening methods, MF/SS patients are HTLV-I seronegative. In recent literature there is some evidence that retroviral genomic information might be integrated into tumor cells without antibodies being detected. We have screened skin biopsies from lesional sites of MF/SS patients from various geographical areas for HTLV-I proviral DNA using the PCR/Southern-blot method with two different primer sets: a primer of the pol as well as a primer of the pX region of the retrovirus, the latter believed to be involved in the neoplastic transformation. Of 27 patients from the state of Ohio, 1 patient showed a positive integration of pol and pX regions, 2 patients only an integration of the pX region, and 1 patient only an integration of the pol region. Of 5 californian patients, 1 had both pX and pol region integration, and 3 showed only a pX integration. One patient from Chile also had pX region integration. None of the eight patients examined from other regions in the U.S.A.and none of the 15 patients of swiss origine were positive. All patients with proviral integration of HTLV-I were suffering from mycosis fungoides. These results indicate that at least in some cases of cutaneous T-cell lymphoma, HTLV-I might be a cofactor in the malignant transformation of the host cell and suggest a geographical clustering.

6

CELL CYCLE CHARACTERISTICS OF CD34+ HEMATOPOIETIC STEM CELLS (HSC) AFTER FEC CHEMOTHERAPY + COLONY-STIMULATING FACTORS (CSF's). Danova M, Rosti V, Venturini M*, Ardizzoni A*, Lucotti C, Cazzola M, Riccardi A. - Medicina Interna e Oncologia Medica, Università e IRCCS San Matteo, Pavia; *Oncologia Medica I, IST, Genova - ITALIA.

To date, most of the studies on the degree and time kinetics of CSF's stimulation, have been concentrated on their in vitro stimulator, role on HSC, while only few data are available in man. We have recently developed an efficient magnetic cell sorting technique to complement multiparameter flow cytometry (DNA content vs bromodeoxyuridine incorporation) (Danova M et al., Blood 82:497,1993) for analyzing some biological properties of HSC. We have utilized this approach to evaluate the kinetic changes of this cell subset after chemotherapy followed by different CSFs. The proliferative effects of G-CSF, GM-CSF (5µg/kg/d x 7 d after CEF) and of GM-CSF + Epo (150 IU/kg x3 times/week)on CD34+ BM cells were investigated in vivo in 18 pts with advanced breast cancer treated with intensified CEF regimen followed by G- (9pts) or GM- (6pts) or GM-CSF + Epo (3 pts).

		LI%	TS (hrs)	Tpot (days)
All the CSF's used				
(and in particular	Control	5.8±3	14.0±3	11.9±2
the combination of	G-CSF	18.6±5	11.5± 2	3.5±3
GM-CSF + Epo)	GM-CSF	19.5±5	10.2 ±3	3.3±2
showed an important	GM-CSF + Epo	23.3±3	9.1±3	3.2±4

effect on the proliferative activity of CD34+ cells (see Table).

The flow cytometric approach utilized is useful for a biological characterization of HSC and can provide information for planning clinical studies combining CSFs with chemotherapy or for optimizing the timing and the techniques of HSC collection for transplantation.

7

HLA-A-LINKED LOCUS D6S265 IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO COMMON ACUTE LYMPHOBLASTIC LEUKAEMIA
Dorak MT, Owen G, Webb D, Worwood M, Burnett AK.
Departments of Haematology and Child Health, University of Wales College of Medicine, Cardiff, U.K.
The homozygosity rate for the common subtype of HLA-A3 was determined by PCR analysis of D6S265 in 38 common ALL patients from South Wales. The 124bp allele of this microsatellite locus correlates to HLA-A*0301. Two cALL patients were homozygous for this allele. In controls (n=144), this genotype was not found despite the presence of two serologically HLA-A3 homozygous subjects. These two were heterozygous for the two serologically indistinguishable HLA-A3 subtypes. These results reveal a relative risk of 19.8 (p=0.01;95% CI=2.0 - 195.8). There was no increase in heterozygous frequency. These findings showed the relevance of homozygosity for only one subtype of HLA-A3 in increased susceptibility to cALL. The lack of an absolute association and the presence of HLA-identical healthy siblings suggest that the major histocompatibility complex is acting as one of the interacting factors in leukaemogenesis.

8

HOMOZYGOSITY FOR HLA-A*0301 REVEALS A HIGH RISK FOR YOUNG-ONSET CHRONIC MYELOID LEUKAEMIA (CML)
Dorak MT, Lee-Jones L, Mills KI, Burnett AK.
Department of Haematology, University of Wales College of Medicine, Cardiff, U.K.

In mice, the major histocompatibility complex influence on carcinogenesis is mainly on the age of onset. We have shown by RFLP analysis that two independent homozygous genotypes (HLA-DR53/DQ3 and -A3) occur only in the young-onset form of CML (Dorak et al. in press). In the present study, PCR analysis of the HLA-A-linked microsatellite locus D6S265 was performed. All patients and controls were from the West of Scotland. Four patients in the early-onset group (age less than 35, n=35) were homozygous for the 124bp allele that correlates to the common subtype (0301) of the serological specificity HLA-A3. There was no homozygous patient in the late-onset group (n=71). In controls, there were three homozygotes (n=228). Heterozygosity for the same allele was not different in patients and controls. The relative risk for early-onset CML conferred by HLA-A*0301 homozygosity was 9.2 (p=0.001, 95% CI=2.3-36.1). These results confirmed our previous findings obtained by RFLP analysis.

9

OVEREXPRESSION OF THE p53 TUMOR SUPPRESSOR GENE IN MYELOID LEUKEMIC K562 CELLS, **Ehinger M, Olsson I and Gullberg U. Dept. of Hematology, University of Lund, Lund, Sweden**

Mutations of the p53 tumor suppressor gene are involved in the development of cancer. p53 was initially characterized as a protooncogene but it was later shown that all p53 proteins with transforming properties were mutant forms. Wild type p53 protein has no transforming properties and overexpression of wild type p53 in some cell lines lacking endogenous p53 suppresses the malignant phenotype. We have overexpressed wild type p53 in K562, a human leukemic cell line in order to investigate the consequences for growth and differentiation. After transfection with the expression vector pc53SN3 coding for human wild type p53 protein, stable subclones were characterized for expression of p53 protein by biosynthetic labeling and immunoprecipitation with the monoclonal antibodies Pab421, Pab1801, DO-1 (binding to wild-type and mutant human p53), Pab1620 (specific for wild-type human p53) and Pab240 (selective for some mutant forms of human p53). Absence of reactivity with the mutant specific antibody Pab240 suggests that the expressed p53 was of wild type conformation. When assayed for proliferation in suspension culture, the growth rate of transfected cells did not differ from that of control cells. Nor was the capacity for clonal growth in semisolid medium significantly different between p53-clones and control cells. The effect of sodium butyrate, all trans retinoic acid, interferon-gamma on clonogenic growth was also similar in transfected clones and in control cells. However some of the transfected clones were more sensitive to clonal growth inhibition by TNF than control cells. This difference in sensitivity to TNF did not seem to depend on a changed sensitivity for induction of apoptosis. Our results suggest that overexpression of wild type p53 in this cell line lacking endogenous p53 does not necessarily diminish the proliferative capacity of the cells but that p53 may be involved in TNF-induced inhibition of growth.

10

MOLECULAR EPIDEMIOLOGICAL STUDY ON ETHYLENE EXPOSED POPULATION.

Ember I., Kiss I., Dept. Public Health,
Univ. Med. Sch., Pecs, Hungary

In a County Hospital in Hungary 91 persons were exposed by ethylene oxid since 1976 till 1992.
Measured level of ETOX was 20-260 mg/m^3 range in workplace area, instead of limited 1 mg/m^3. Among exposed patients 8 tumours has been detected (5 breast, 1 ovary, 1 colon, 1 astrocytoma).
We have investigated 20 exposed persons from all of them. DNA arising from peripheral white blood cells, and nucleic acid was slot blotten onto Hybond filter hybridized with EC labelled cloned oncogene probe.
Both oncogene (N-ras and C-myc) were detected higher signal on autoradigrams. It looks like these oncogenes are amplified not it tumours patients but in exposed not tumorous persons too.

Key worlds: ETOX, ONCOGENES, EXPOSITION.