Lym 08**CLONAL REARRANGEMENTS OF T-CELL RECEPTOR AND IMMUNOGLOBULIN GENES AS SENSITIVE MARKER FOR BONE MARROW INVOLVEMENT IN NON-HODGKIN'S LYMPHOMA**

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The clinical stage of patients with Non-Hodgkin's Lymphoma (NHL) not only is a prognostic parameter but also has important implications with regard to the therapeutic protocol. As to the staging of NHL, the involvement of the bone marrow indicates early dissemination of disease. However, the detection of small numbers of infiltrating neoplastic T- or B-cells often provides great difficulties because diagnostic procedures including immunophenotyping are not specific to transformed lymphocytes. Recently, it has been shown that rearrangements of T-cell receptor (TCR) and immunoglobulin (Ig) genes can be used to identify tumors of T- and B-cell lineage and to demonstrate monoclonality. We have used cDNA probes for the TCR B-chain and Ig heavy chain J region genes to document clonal expansions of neoplastic T- or B-cells in the bone marrow of select cases, where histological and cytological examination was inconclusive. In four of six patients Southern blot analysis of bone marrow DNA revealed rearranged TCR or Ig genes indicating the presence of T- and B-cell tumors, respectively. Rearrangements were never detected in the bone marrow of healthy donors or patients with CML. In this study, we demonstrate that the detection of clonal TCR and Ig gene rearrangements unequivocally documents bone marrow involvement in NHL patients. These unique DNA rearrangements promise to be useful for confirming the pathological diagnosis and for assessing the stage of NHL.

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Lym 09**TRANSPLANTATION ANTIGENS OF CLASS II, STRUCTURE, FUNCTION, AND THEIR MONOCLONAL ANTIBODIES**

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Transplantation antigens of class II of the human homozygous lymphoblastoid B-cell line H2LCL were isolated by preparative biochemical procedures (G. Egert, Hoppe-Seyler's Z. Physiol. Chem. Bd. 365, S. 1291-1308, 1984). The amino acid sequence of the HLA-DR2 antigen was determined using micro-methods. It consists of two noncovalently associated subunits: an α chain of molecular weight 34 kD (229 amino acid residues) and a β chain of 29 kD (237 amino acid residues). The membrane glycoproteins are organized in three parts: first, the long N-terminal part encompassing 191 and 198 amino acid residues of the α and β chain, respectively; second, hydrophobic regions comprising 23 residues in both heavy (α) and light (β) chain which penetrate the cell membrane and third, a basic hydrophilic short C-terminus of 15 and 16 amino acids immersing into the cytoplasm. The higher molecular weight of the α chain originates from two N-glycosidically linked side chains in contrast to only one carbohydrate moiety attached to the extracellular part of the β chain. The determination of the nearly complete amino acid sequence of the DR2 α chain as well as the characterisation of at least 7 class II β -chains provides an insight into the isotopic complexity of human class II antigens. In addition 4 monoclonal antibodies (moAb) were prepared and characterized. MoAb 35.12 and moAb 43.7 specific for p 34 proved to be different after 2D separation of p 34. After IEF of p 34 moAb 43.7 proved to react with all α chains, whereas moAb 35.12 yielded a reaction only with a single α chain. MoAb BE 11.10 and BE 44.23 proved to be specific for p 29; moAb BE 11.10 showed reaction with a single band of p 29, moAb 44.23 reacts with two bands of p 29 after IEF. MoAb 44.23 additionally reacts with a well defined peptide of the DR2 β chain, in position 22-36. Comparison of sequences of other chains showed distinct differences in their isotypes specifically in this region. Medizinische Universitätsklinik, Hugstetter Straße 55, D-7800 Freiburg (BrsG.)

Lym 10**CHEMOTHERAPY OF HUMAN LYMPHOMA CELL LINES AFTER HETEROTRANSPLANTATION IN NUDE MICE**

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Four malignant human hematopoietic in vitro cell lines (Hodgkin's disease derived lines L 428 and L 540, Burkitt's lymphoma line BJAB, lymphoblastic lymphoma line L 735) and a diploid, Epstein-Barr virus transformed lymphoblastoid cell line of cord blood origin were transplanted into the brain (i.c.) of nude mice as well as into the muscle (i.m.) in a parallel experiment. After the tumors had developed, they were treated by intraperitoneal application of a panel of cytostatic drugs given as monotherapy. Parameters of treatment success were reduction of tumor size (i.m.) and prolongation of survival time (i.c.) compared to an untreated control group. Therapy results in both transplantation routes were analyzed and compared using the Student's t-test. Sensitivity and resistance of the cell lines tested in the i.c. system were in good accordance with results in the i.m. system. Treatment of the T-lymphoma line L 735 with cyclophosphamide (CY) resulted in complete remission of i.m. tumors in several cases. Repetitive treatment with CY induced decreased sensitivity to CY in the L 735 and the Hodgkin line L 540. After recultivation of L 540 CY tumor cells in vitro cytogenetic analysis demonstrated a new structural chromosomal marker. We conclude that the intracerebral transplantation system is useful as a model in the cytostatic treatment of human neoplastic cells which are able to proliferate only in the immunologically privileged site of the nude mouse brain. The mutagenic influence of CY resulting in sub-lines with diminished sensitivity and more aggressive growth behavior as well as the effect of optimized timing of drug application on tumor reduction will also be discussed.

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Lym 11**MYC-ONCOGENE EXPRESSION IN B-CELL CHRONIC LYMPHATIC LEUKEMIA (B-CLL) AND IN HAIRY CELL LEUKEMIA (HCL)**

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Previous studies have demonstrated that cells of B-chronic lymphatic leukemia (B-CLL) develop both phenotypic and biochemical characteristics of hairy cell leukemia (HCL) when incubated with phorbol ester (TPA). We have investigated the myc-1-oncogene-expression in B-CLL and in HCL, the change in enzyme pattern and expression of the Tac-antigen of B-CLL after incubation with TPA. 6 patients with B-CLL, 6 patients with HCL and 3 controls were studied. The B-CLL cells were incubated with or without (controls) 2 nM TPA at 37°C for 96 hours. Expression of the myc-1-oncogene was tested in HCL and in white blood cells from healthy donors. Concerning the myc-1-oncogene expression no difference could be seen between HCL and controls. However, in 3 out of the 6 cases of B-CLL, myc-1-expression was 4-5 fold higher than in HCL and in the controls. After treatment with TPA an increase in cells positive for tartrate resistant acid phosphatase, in Tac-antigen expression and also in purine nucleoside phosphorylase (PNP) activity could be found, indicating a phenotypic change from a B-CLL to a HCL pattern. The myc-1-oncogene expression, however, remained unchanged, so that myc-1 was further overexpressed in 3 cases of B-CLL. Conclusions: 1. HCL is characterized by a myc-1-oncogene expression similar to white blood cells from healthy controls. 2. In 3 of the 6 cases of B-CLL, an overexpression of myc-1-oncogene was found. 3. Incubation of B-CLL with TPA causes changes in Tac-antigen, in TRAP expression and in PNP similar to the characteristics of HCL. However the overexpression of myc-1 is not altered by incubation with 2 nM TPA. Thus only "HCL-like" cells but not true HCL could be obtained from B-CLL by incubation with TPA.

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