1. Diagnostic / Pathology

THE ROLE OF GALLIUM-67 SCAN (GA) IN STAGING AND RESTAGING PATIENTS WITH HODGKIN'S DISEASE (HD).
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Introduction: The role of GA versus conventional imaging procedures in staging and restaging HD is still controversial.

Patient Characteristics: From 990 to 994, 125 pts treated with combined ABVD (77) or VEBEP (VP16, Epidophorbine, Bleomycin, Cytoxan, Prednisolone) (48) both followed by radiotherapy (RT) were submitted to GA, lymphography, CT scan ± NMR before and after the chemoradiotherapy program.

Disease extent was as follow: CS I-IIA: 77 pts.; CS IIb-IIb+IV: 48 pts.; nodal involvement: 95 pts. extra ± nodal: 30 pts.; mediastinal disease: 111 pts.; supra-diaphragmatic nodal sites (SDNS): 334; nodal sites below the diaphragm (NSDB) : 44.

Results: Gallium uptake was observed in 82% of SDNS (90% at mediastinum), in 43% of NSDB, in 75% of lung lesions, and in 100% of bone lesions, respectively. Ninety-nine and 5 of GA positive SDNS became negative (94% at mediastinum) after treatment. Comparing GA to CT scan in detecting initial or residual mediastinal disease, the main results were as follows:

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Out of 12 relapses, 7 occurred within 12 months: 3 with and 4 without GA uptake at restaging. The other patients, all with GA negative at restaging, progressed later or in sites below the diaphragm.

Conclusions: At staging, GA is comparable to CT scan in detecting mediastinal involvement while CT scan remains the standard procedure in detecting abdominal disease. At restaging, GA seems to be superior to CT scan in predicting complete remission of mediastinal adenopathy with a specificity of 97%, while a negative GA does not predict late relapses which may occur in other sites.

WHOLE BODY POSITRON EMISSION TOMOGRAPHY (PET) IN PATIENTS WITH LYMPHOMAS FOR DIAGNOSIS OF RESIDUAL MASS
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Objectives: PET using 18F-fluorodeoxyglucose (FDG) may offer the possibility to differentiate sites of vital from necrotic residual masses. To assess the value of PET-17 patients (pts) with Hodgkin's Disease (HD) and 17 pts with Non-Hodgkin's lymphoma (NHL) underwent FDG-PET following therapy. In all these pts residual disease was suspected after completion of therapy.

Patient characteristics and methods: Staging was assessed by routine methods including laboratory screening, computed tomography of thorax and abdomen, sonography, bone biopsy, if indicated liver biopsy and magnetic resonance tomography in pts with bone or soft tissue involvement. At diagnosis 7 pts presented with stage I, 13 with stage II, 5 with stage III and 9 with stage IV.

Median age was 43 (21-73) years. A total dose of 250-400 MBq FDG was injected i.v. and whole body PET was performed 30-45 minutes later.

Results: 2 pts achieved CR according to routine staging. Residual mass was found in 32 pts with routine methods. FDG-PET was negative in 17 pts. They were considered to be in CR. None of them relapsed after a median follow-up of 36 weeks (4-72 weeks). FDG-PET was positive in 17 pts. 16 pts had residual mass with routine methods. 4 pts received radiation after PET. Their median follow-up is 28 weeks (16-44) without relapse. 2 other pts with lasting CR (72 and 32 weeks) had FDG-uptake outside the residual mass - 1 pt in the lung with confirmed pneumonia, 1 pt nodular pleural uptake of unknown origin. 3 pts had histologically confirmed lymphoma. 1 pt relapsed histologically confirmed 65 weeks after PET, and 2 additional pts relapsed according to routine methods 43 and 40 weeks after positive PET. The remaining 4 pts have a follow-up of 1, 2, 24 and 28 weeks. The last 2 pts are likely to be false positive because of fracture (left humerus) and a coxitis at the lymphoma site. 6 of 10 pts with FDG-uptake in the residual mass after completed therapy relapsed. According to radiological routine restaging 2 pts achieved CR but were clinically suspicious for persistence. 1 pt was negative in PET too. In 1 pt an additional focus was found in the humerus with PET in spite of normal bone scintigraphy with histologically confirmed lymphoma. There were no false negative results. 2 false positive results inside the residual mass, 2 false positive results outside the residual mass and 2 pts with a very slight follow-up.

Conclusion: PET performed for evaluation of residual disease after treatment of lymphoma has a high predictive value.

MAGNETIC RESONANCE IMAGING OF FEMORAL MARROW IN PATIENTS WITH MALIGNANT LYMPHOMA
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Magnetic resonance imaging (MRI) of femoral marrow was studied in consecutive 56 patients with newly diagnosed lymphomas (48 non-Hodgkin's lymphoma and 8 Hodgkin's disease). MRI appearance of femoral marrow was obtained by T1-weighted spin echo method and short T1 inversion recovery (STIR) technique. Of 56 patients, 29 patients (52%) showed abnormal images on their femoral marrow MRI. Twelve patients (41%) of them revealed negative findings of BM biopsies. All patients with positive bone marrow (BM) biopsy results had an abnormal signal intensity on their femoral marrow MRI. Three patients of femoral marrow MRI, namely scattered (72%), uniform (21%), and nodular (7%) pattern were noted. It seemed unlikely, that histology, immunophenotype, serum LDH level, and hemoglobin level could account for these particular patterns of femoral marrow displayed by their MRI. Patients with abnormal patterns on their femoral marrow MRI had a significantly worse overall survival when compared with those with normal MRI pattern (p=0.0129). Furthermore, patients who showed abnormal patterns on their femoral marrow MRI despite normal BM histology had a significantly poorer survival when compared with those who showed normal MRI findings (p=0.016). This study indicates that MRI of femoral marrow was shown to be a sensitive tool of detecting lymphomatous BM involvement, and that patients who showed abnormal images on their femoral marrow MRI should be treated intensively.

FDG PET SCANNING IN STAGING AND REMISSION ASSESSMENT OF MALIGNANT LYMPHOMA
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As part of an on-going programme to improve the accuracy of pre-treatment staging and remission assessment for malignant lymphoma we have been using 2-(2-Fluoro-2-deoxy-β-D-glucose) (FDG) whole body positron emission tomography (PET) in conjunction with computed tomography (CT). FDG has consistently been shown to concentrate preferentially in metabolically active tissue including malignant lymphoma.

83 adult patients seen in the Clinical Oncology Department at St Thomas’ Hospital between January 1993 and July 1995 who have undergone an FDG whole body PET scan form the basis of this review. 42 patients had FDG PET scan performed at the time of initial diagnosis, 15 with Hodgkin’s Disease (HD) and 27 with Non-Hodgkin’s Lymphoma (NHL). Post treatment PET scans have been performed in 26 patients.

FDG PET scanning demonstrated more sites of active disease in both HD and NHL when compared with other clinical or CT evaluation. FDG uptake was demonstrated independent of HD subtype or NHL grade. There were no false ‘positive’ scans with a false negative rate of less than 2% in a total of 130 PET scans. The management of 10 patients was changed by the information obtained from the FDG PET scan. FDG PET scanning appears to be a useful marker for residual disease activity following treatment in malignant lymphoma however follow-up on many of the patients is relatively short and definitive evidence of its value must await more prolonged observation.

This retrospective review indicates that FDG PET scanning is an effective and accurate technique for detection sites of initial involvement by lymphoma and for monitoring response to treatment and relapse. A prospective study to assess the impact on management and treatment outcomes will be necessary to confirm these findings and evaluate cost effectiveness.

1. Diagnostic / Pathology

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WHOLE BODY POSITRON EMISSION TOMOGRAPHY (PET) IN PATIENTS WITH LYMPHOMA: A NEW METHOD FOR STAGING AND DETECTING EXTRANodal LESIONS.
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Despite use of computed tomography (CT) and MRI exact staging in patients (pts.) with lymphoma is very difficult, because "whole body" MRI or CT is routinely not practicable. Exact staging is however mandatory in pts. with lymphoma as therapy depends on different stages. With PET after injection of 2-[18F]fluoro-2-deoxy-D-glucose (FDG) we have a new method which can be used to visualize metabolically active tissue. One question was asked: Would whole body FDG-PET improve the staging? 53 pts. (26 pts. with Hodgkin's disease and 27 pts. with Non Hodgkin's lymphoma) underwent FDG-PET as part of the staging. A total dose of 200-400 Mq FDG was injected i.v. Pts. also underwent CT of thorax, abdomen and pelvis and bone marrow biopsy. If necessary bone scanning and MRI was performed. FDG-PET was positive in all areas known to be involved by routinely practised methods in 40/53 pts. in 1/53 patient splenic involvement was not detected by FDG-PET. In 2/53 pts. additional lesions were detected by FDG-PET, which could not been proven as true positive. In 10/53 pts. PET identified additionally lesions (2,liver, 6,skeleton, 2,spleen) which were not detected by routinely practised methods. In 5/10 pts. MRI was performed after positive PET-FDG-PET and lesions could be proven as true positive. In 3/10 pts. bone scanning was necessary for detecting the lesions described by PET. In one patient skeleton was proven as positive by biopsy (bone scanning and MRI were negative). In one case a laparatomy was performed after positive PET-FDG-PET for liver involvement (negative on CT, MRI and ultrasound). 4 pts. were upstaged from stage II to III and 6 pts. from stage II to IV. Whole body FDG-PET has a sensitivity of 99% if performed for staging. It seems to be a very sensitive non invasive method to visualize extranodal lesions or spleen involvement in pts. with lymphoma. FDG-PET will turn out to be the most reliable non invasive method.

DISTRIBUTION OF THE DIFFERENT ENTITIES RECOGNIZED IN THE REAL CLASSIFICATION AMONG 695 CONSECUTIVE PATIENTS DIAGNOSED OF LYMPHOID NEPLASMS IN A SINGLE CENTER

Results: B-cell Neoplasms 542 (77.3%), T-cell pre-Bymphoblastic leuk., lymph. 18 (2.6%), II-Peripheral B-cell neoplasms: 1-cell CLL/small lymphocytic lymph. Jnr. leuk. 144(20,7%) 2-cell lymphomacytoid lymph./immunocytoxia 221(3,2%) 3-Mantle cell lymph. 16 (2.3%) 4. Follicle center lymphoma 50(7,2%) 5.Marginal zone B-cell lymph. 284(0,6%) 6.Splenic marginal-zone lymph. 2(0,3%) 7. Hairy cell leuk. 50(7,0%) 8.Plasmacytoma/plasma cell myeloma 100(14,4%) 9.Diffuse large B-cell lymph. 108(15,5%) 10.Burkitt's lymph. 1(0,1%) 11.High grade B-cell lymph. Burkitt-like 3(0,4%) B-cell lymph. unclassified according to REAL 45(6,5%) T-cell and putative NK-cell Neoplasms 68 (9,4%) Precursor T-lymphoblastic leuk./lymph. 111(1,6%) II-Peripheral T-cell and NK-cell neoplasms 1.T-cell CLL/Jr. leuk. 0(0,0%) 2.Large granular lymphocyte leukemia 0(0,4%) 3.Mycosis fungoides/Sézary syndrome 20(2,9%) 4.Peripheral T-cell lymph. unspecified 121(7,7%) 5.Large granular lymphocytic T-cell lymph. 0(0,0%) 6.Angioimmunoblastic T-cell lymph. 0(0,0%) 7.Intestinal T-cell lymph. 2(0,3%) 8.Adult T-cell lymph. /leuk. 0(0,0%) 9.Anaplastic large cell lymph. CD30 + 8(1,2%) 10.Anaplastic large cell lymph. Hodgkin's like 0(0,0%) T-cell Lymphoma unclassified according to REAL 20(3,0%) Hodgkin's Disease 77(11,0%) I-Lymphocyte predominance 0(0,0%) II-Nodular sclerosis 41(7,3%) III-Mixed cellularity 17(2,4%) IV-Lymphocyte depletion 20(3,0%) V-Lymphocyte-rich classical HD 110(1,5%) * Hodgkin's Disease unclassified according to REAL 12(1,7%) ** Lymphoid neoplasms unclassified according to REAL 111(1,6%)

MANTLE CELL LYMPHOMA: A CLINICOPATHOLOGIC ANALYSIS OF 80 CASES.

Mantle cell lymphoma (MCL) is an uncommon yet distinct subtype of malignant lymphoma. Characteristics of its clinical and pathologic features is limited by the small number of cases published to date. This study examines 80 cases of MCL seen at our institution over the past 8 years: 70% were male and 30% female with a median age of 65 years. Median overall survival (OS) was 43 months. The majority (87%) presented with advanced stage disease confined to lymph nodes in 75% with diffuse involvement in the remaining 25%. 25% had extranodal lesions in the peripheral blood and bone marrow involvement during the course of their disease. 6% had multiple lymphomatous polyposis. Prognosis was not significantly affected by age (< 60 yr, stage LD vs III/IV, performance status (PS) 0 vs 1-2) or LNI (normal vs increased). All cases were diagnosed using routine histology with ancillary immunophenotypic, cytogenetic and/or molecular genetic studies available in 57% MCL architecture was diffuse in 76% of cases, nodular but lacking a true mantle-zone (MG) pattern in 16%, and classic MG pattern in 6%. The OS among these groups was indistinguishable, but the number of MG cases was small. Mitotic score (MS), defined as the number of mitotic figures in 10 high power fields (400x), had prognostic significance. MS of >20 (78%) was associated with significantly longer OS than was a MS of >0 (22%) (median OS = 32 vs 15 months, 

p53 MUTATIONS AND CYCLIN-DEPENDENT KINASE INHIBITOR P16 INK4A AND P18 INK4B, CDKN1A, CDKN1B, CDKN1C IN MANTLE CELL LYMPHOMA: ASSOCIATION WITH EXTRANODAL DISEASE AND BLASTIC MORPHOLOGY.
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Mantle cell lymphoma (MCL) is characterized by cyclin D1 (BCL1, PRAD1, CCND1) gene rearrangement in > 70% of cases and overexpression in virtually all cases, suggesting a role in pathogenesis. However, overexpression of this G1 cyclin alone has not been lymphomagenic in transgenic models. To test the hypothesis that loss-of-function mutations in cell cycle negative regulatory elements coupled with cyclin D1 overexpression may synergistically contribute to lymphomagenesis, we analyzed p53 point mutations and p16/p15 deletions in MCL and low-grade B-cell NHL and CLL. p53 mutations were analyzed by immunohistochemistry of fixed paraffin embedded tissue and by PCR amplification and direct sequencing of p53 exons 5-8, p15 and p16 gene deletions were detected by signal specific control relative to hybridizations on Southern blot analysis using cDNA probes (provided by D. Beach). 54 MCL (39/72%) with ICR1/ cyclin D1 rearrangement, 3 lymphomas with villicus lymphocytes, 15 CLL/small lymphocytic lymphomas and 4 plasmatycyloid lymphomas were studied.

Five MCL had p15/p16 deletion and 3/22 had p53 mutation; none showed both p33 mutation and p15/p16 deletion. No abnormalities were found in non-MCL cases. p53 mutations occurred in cyclin D1 (codon 277), exon 7 (248) and exon 5 (178). 6/8 MCL (75%) with p15/p16 deletion or p53 mutation had extranodal disease (lung, GI, prostate, or soft tissue) vs. 25% of MCL without these abnormalities. 3/8 MCL (38%) cases with p16/p15 deletion histologically had p16/p16 deletion, vs. only 2/48 (4%) non-MCL cases. These findings support the hypothesis that at least a subset of MCL may result from increased cyclin D1 expression coupled with additional abnormalities in tumor suppression regulatory cascades. The results also suggest an association with extranodal disease and blastic morphology. Further studies are required to identify loss of tumor suppressor genes in cases without p15/p16 or p53 abnormalities.
CD 5-POSITIVE DIFFUSE LARGE B CELL LYMPHOMA (DLBL): CLINICAL CHARACTERISTICS AND TREATMENT OUTCOME

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Departments of Medicine and Pathology, Fujiwara Health University, Departments of Medicine and Pathology, Aichi Cancer Center Hospital, Aichi, Japan.

Cellular and histologic characteristics of the CD5+ DLBL which constitutes a small fraction of DLBL were described (Matolcsy et al., Am J Pathol 147, 207, 1990). This is a report on its clinical characteristics and treatment outcome based on 16 patients (pts). Median age was 71 years (range 22-84) with M/F ratio 5/11, clinical stage IB/II in 2 pts and III/IV in 14. Twelve had B symptoms. Extramedullary sites of involvement included Waldeyer's ring (4 pts), bone marrow (4), liver (2), stomach (2), PS 0, 1 in 9 and 2,3,4 in 7. Serum LDH was elevated in 12. Four had international index I/LI risk disease and 12 had II/I disease. Immunohistochemically, cyclin D1 was negative in 15/16 pts tested, and BCL2 was positive in 3/7 tested. Eleven out of 16 responded to CR to various treatment regimens. 7/11 (64%) relapsed, 6 of them within 12 months of CR. DFS was 20% and OAS 31% at 3 years. CD5+ DLBL may constitute a subtype with advanced disease on diagnosis, fairly good response to treatment, but early relapse.

SÉZARY SYNDROME: T-CELL CLONES DISPLAY T-HELPER 2 CYTOKINES AND EXPRESS THE ACCESSORY FACTOR 1 (INTERLEUKIN-2 RECEPTOR β CHAIN)

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Sézary Syndrome (SS) is a leukemic variant of low-grade cutaneous T-cell lymphomas. The clonal T-cells in this lymphoproliferative disorder are poorly characterized. Using antibodies against the variable region of the T-cell receptor (TCR) (αβ chain) we have identified four predominant T-cell clones (2 Vβ6+ clones, 1 Vβ5.1+), 1 Vβ2(a)+) in SS patients' peripheral blood mononuclear cells (PBMC). Their phenotype was CD4+, CD4+, CD5+, CD45RO+. Clonal T-cells were purified and cytokine transcription and secretion was analysed by RT-PCR followed by hybridization with biotinylated probes and ELISA. The IL-10 PCR product was cloned and sequenced and found to be identical to the published cDNA sequence. The presence of the accessory factor 1 (AF-1) (or IFN-γ receptor β chain) encoding mRNA was assessed by RT-PCR and immunostaining using serum of rabbits immunized with the extracellular domain of a recombinant human AF-1 protein followed by APAAP staining. Clonal T-cells transcribe and secret mainly T helper 2 cytokines (Interleukin-10, -5, -13). mRNA from purified SS clones but not from mRNA of SS total peripheral blood mononuclear cells (PBMC) was positive for AF-1 in an agarose gel and after hybridization. AF-1 transcription was associated with membrane-bound immuno-reactivity for AF-1 in SS clones. SS derived T-cell clones display T helper 2 cytokines. This weakens cell-mediated immunosurveillance and explains clinical and immunological abnormalities in SS patients. The T helper 2 cytokine spectrum is associated with the overexpression of AF-1.
2. Biology

Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity

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Anaplastic large cell lymphoma (ALCL) is a subtype of non-Hodgkin's lymphoma (NHL) characterized by the CD30+ large neoplastic cells and sometimes carries a (12;5)(p13;q35). Recently, we found a novel hyperphosphorylated 80-kDa protein tyrosine kinase, p80, in ALCLs with t(12;5). Subsequent cDNA cloning revealed p80 to be a fusion protein of two genes, the novel tyrosine kinase gene and the nucleophosmin gene, in accordance with the sequence of the NPM/ALK gene (Sailor et al.). We also showed that overexpression of p80 in NIH3T3 cells induced neoplastic transformation, suggesting that the p80 kinase seems to take primary role in tumorigenesis. Meanwhile, the clinicopathologic features of p80-carrying ALCLs have remained unclear. Thus, paraffin sections of 105 cases of ALCL were immunostained using anti-p80 antibody, and their clinicopathologic characters were analysed. Thirty out of 105 ALCLs were shown to express p80. clinicopathological comparison between p80-positive and -negative ALCLs revealed that p80-positive cases occurred in a far younger patient age group (16.2±12.9 yr; p80-negative cases, 51.6±22.3 yr; p<0.0001) and favors a far better 5-year survival rate (79.8%; p80-negative group, 32.9%. p<0.01). These data showed that p80-positive ALCL is a distinct entity both clinically and pathologically, and should be differentiated from p80-negative ALCL. We are now investigating the abnormal signal transduction pathway induced by this oncogenic protein p80.

Detection of anaplastic large cell lymphoma (ALCL) specific translocation t(2;5) in cases of cutaneous lymphoproliferations

ALCL of the skin, lymphomatoid papulosis and pleomorphic medium and large cell T-cell lymphomas show many similarities to nodal anaplastic large cell lymphoma and Hodgkin's disease by means of morphology, phenotype, genotype. While the detection of the translocation t(2;5) (p23;q35) is frequently found in nodal cases of ALCL of D- and T-cell type, only limited information is available on the detection of t(2;5) in cases of cutaneous lymphoproliferations.

We investigated for the aberrant expression of the translocation specific protooncoprotein (alk) by RT-PCR analysis with different primers. Amplified products were either sequenced or confirmed by Southern-blotting and for some cases immunohistochemistry with a breakpoint specific antisense primer prepared in our laboratory was prepared. Both, positive and negative cases were observed. For some cases, subsequent biopases were investigated, showing that sometimes the primary lesion is negative, while the secondary biopsy showed a translocationspecific product. The immunohistochemical analysis revealed positivity of Hodgkin-and Sternberg-Reed-like cells in a number of those cases which were already shown to be positive by RT-PCR analysis. t(2;5) translocation results in a fusion gene (npm/alk) with tyrosine kinase activity. This tyrosine kinase which is normally silent in lymphocytes may be at least in part responsible for the cellular transformation and for the pathogenesis of t(2;5)+ neoplasias. The detection of t(2;5) in different entities of cutaneous lymphoproliferations (ALCL, LYP and pleomorphic T-cell lymphomas) underlines the biological relationship of these entities. Further studies may evaluate the usefulness of the detection of t(2;5) for the prediction of clinical course.

THE USE OF P.5.H. IN THE DETECTION OF t(12;5)(p23;q35): TRANSLLOCATION IN ANAPLASTIC LARGE CELL LYMPHOMA

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Anaplastic large cell lymphoma (ALCL) is a recently recognised, distinctive type of non-Hodgkin's lymphoma characterised by atypical large cell cytology and expression of a member of the TNF receptor superfamily, CD30. A characteristic chromosomal translocation has been identified in ALCLs of T-cell or null lineage which juxtaposes a novel tyrosine kinase (anaplastic lymphoma kinase, ALK) located at 2p23 and the nucleophosmin gene (NPM) located at 5q35. Although not present in all ALCLs the t(12;5)(p23;q35) appears highly specific for this form of lymphoma. We have employed the Fluorescence In-Situ Hybridisation (FISH) technique to demonstrate the t(2;5) using whole chromosome paints for chromosome 2 (Cambridge catalogue number 1662B1) labelled with NHS and Texas Red and for chromosome 5 (Oncor; catalogue number PS259) labelled with Digoxigenin and FITC. We have also used two YAC probes for chromosome 2 labelling at 2p16 (D2S132) and at chromosome 2p24 - 25 (D2S311). A chromosome 5q35 YAC probe containing the proto-oncogene phosphatase encoded by the cyclin 104 gene was also utilized.

We have applied these probes to cytospin preparations of the Karpas 299 cell line known to possess the t(2;5), to imprint preparations of 4 cases of ALCL which had been shown to contain the t(2;5) by classical cytogenetics and to 4 cases of ALCL which did not contain t(2;5) by classical cytogenetics.

Reverse transcriptase PCR was performed using the 5' NPM and 3' ALK primers as described by Morrison et al. (Science 261: 1281. 1993) on 2 cases in which we had snap frozen material, one possessing t(2;5) and the other lacking this translocation by classical cytogenetics.

Immunohistochemical staining of paraffin embedded material was performed using p80 antibody directed against the ALK protein in 4 cases, 2 possessing t(2;5) and 2 without the translocation. The FISH technique using either the 2 and 5 chromosome paints or the YAC probes was able to detect the t(12;5)(p23;q35) in the Karpas 299 cell line and in all 4 cases of which cytogenetics analyses had revealed the translocation. The translocation was difficult to detect in the lymphoma cell line and in metaphase spreads. None of the 4 cytogenetically negative cases demonstrated t(2;5) by FISH. There was complete correlation with t(2;5) as demonstrated by FISH and positive immunostaining for p80 protein. RT-PCR demonstrated the NPM-ALK YAC hybrid RNA in the single case positive for t(2;5) by FISH study; hybrid RNA was absent from the negative case.

FISH appears to be a sensitive and reliable technique for the demonstration of t(12;5)(p23;q35) and correlates well with other methods of detecting this translocation.

CHARACTERIZATION OF 22 CASES OF MARGINAL ZONE B-CELL LYMPHOMA WITH TRISOMY 3


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Trisomy 3 represents the most frequent and consistent chromosomal abnormality characterising the recently defined entity marginal zone B-cell lymphoma (MZBCL). By cytogenetic analysis and/or fluorescence in situ hybridization (FISH) on interphase nuclei we found an increased copy number of chromosome 3 in 22 of 36 (61%) successfully analysed cases, including 8 of 12 cases with extranodal MZBCL, 8 of 13 cases with nodal MZBCL and 6 of 11 patients with splenic MZBCL. Sensitivity of interphase cytogenetics was somewhat higher than that of conventional cytogenetic investigations. Structural chromosomal changes involving at least one chromosome 3 were seen in 11 of the 20 cases with an increased copy number of chromosome 3: +cel(3)(p13) was demonstrated in 3 cases, in one of them even as the sole chromosomal aberration; +t(6;9)(p10) was seen in two other patients; and rearrangements involving various breakpoints on the long arm of chromosome 3 were found in the remaining cases. FISH on metaphase spreads confirmed these structural abnormalities and additionally showed two unexpected translocations involving chromosome 3. We conclude that (1) trisomy 3 occurs in a high proportion of extranodal, nodal, and splenic MZBCL; (2) FISH on interphase nuclei is an additional and sensitive tool in detecting an increased copy number of chromosome 3 in MZBCL; (3) additional structural abnormalities involving the long arm of chromosome 3 are frequent but non-recurrent and are perhaps secondary changes; and (4) abnormalities such as +cel(3)(p13) and +t(6;9)(p10) suggest that genes located on the long arm of chromosome 3 are of particular importance in the pathogenesis of MZBCL.
THE CLINICAL SIGNIFICANCE OF DETECTING CLONAL IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENT IN B CELL NON-HODGKIN'S LYMPHOMA

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Paraffin-embedded biopsies from 57 patients with B cell non-Hodgkin's lymphoma (B-NHL) were analysed by polymerase chain reaction (PCR) for presence of clonal immunoglobulin heavy chain (IgH) gene rearrangements. The clonal IgH gene rearrangements were found in 72% (42/57) of the patients but none of the 23 controls (4 of T-NHL, 8 of non-lymphoid carcinoma, 5 of reactive lymph node and 6 of normal bone marrow). The clonal IgH gene rearrangement used as a gene marker of B-NHL, a sensitive method of polymerase chain reaction amplification in conjunction with single-strand conformational polymorphism (PCR-SSCP) analysis was performed on the stored bone marrow slide specimens from 38 untreated patients with B-NHL for detecting minimal disease. The incidence of minimal disease in bone marrow specimens was 88% (33/38). The patients with minimal disease in bone marrow had a short survival time than those without (p=0.05). Seven of 14 patients at clinical stages I or II were found with minimal disease in bone marrow and would have been upstaged if these results were used for staging. The preliminary results showed that the clonal IgH gene rearrangement was only found in B-NHL and could be used as a gene marker for diagnosis of B-NHL. Detection of minimal disease may provide some important information for assessing dissemination of lymphoma cells and evaluating prognosis of the patients.

PATHOGENETIC AND CLINICAL IMPLICATIONS OF BCL-6 AND BCL-2 GENE MOLECULAR CONFIGURATION IN DIFFUSE LARGE B-CELL LYMPHOMA

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Bcl-6 (ALZ3) and Bcl-2 gene rearrangements have been reported in 20-35% and 15-25% of diffuse large B-cell lymphomas (DLBCL), respectively. Although these lesions have been associated with diverse clinical outcomes (i.e. more favorable in Bcl-6 rearranged and poorer in Bcl-2 rearranged cases), their prognostic significance is still controversial. In this study, we have investigated by Southern blot the Bcl-6 and Bcl-2 gene configuration in a series of 70 DLBCL histologically defined according to the updated IPI classification, and correlated these molecular findings with clinical features at presentation and prognostic outcome. All patients had de novo DLBCL and cases with a previous history of low grade follicular lymphomas were excluded from the analysis. The majority of DLBCL had a centrosomal morphology (56/79 = 71%). Bcl-6 rearrangements were detected in 23/79 (29.1%) cases and were similarly associated with centroblastic (18/56 = 32.1%) or immunoblastic (5/13 = 38.5%) histotype. Conversely, Bcl-2 rearrangements were only detected in two centroblastic lymphomas (2.5%). Compared to Bcl-6-germinal cases, patients with Bcl-2 alterations had a lower incidence of bone marrow involvement (6% vs 22%) and a better response to treatment (complete remission rate = 67% vs 44%, partial response rate = 30% vs 42%) suggesting that the classification of Bcl-6 rearrangements are frequently found in DLBCL. The incidence of Bcl-2 involvement of our series is significantly lower compared to the frequencies reported in other European and North American studies, probably reflecting epidemiological variability and/or heterogeneity in patient selection. From a clinical viewpoint, our findings favour the notion of a better prognostic outcome in cases with Bcl-6 rearrangement, although the differences were not statistically significant. Further studies in larger series of uniformly treated cases are warranted in order to elucidate the clinical significance of Bcl-6 gene configuration in DLBCL.

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COMPETITIVE POLYMERASE CHAIN REACTION TO QUANTITATE t(14;18) CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH FOLLICULAR NON-HODGKIN'S LYMPHOMA.


The detection of residual circulating t(14;18)-carrying cells in peripheral blood of remission patients with low grade follicular non-Hodgkin's lymphoma (FL) has been previously described. However, clinical and biological significance of the t(14;18) cells remains unclear. We have developed a competitive Polymerase Chain Reaction (PCR) to estimate the number of cells in peripheral blood before, during and after treatment of patients with FL. Artificial competitor fragment (CF) was constructed using the microsatellite in the CAT gene, which was modified in order to be amplified with the same primers as the t(14;18) targets. Furthermore, an internal sequence was added for hybridization and quantification. Serial dilution of CF were added to one microgram of DNA sample and amplified for 40 cycles. The two PCR products were separated by gel electrophoresis, transferred to nylon membrane that was subsequently hybridized with a bcl-2 oligonucleotide probe. Autoradiographs were analysed by densitometry scanning. Controls of variability showed small variations allowing to conclude to the good precision and reproducibility of the method. Accuracy was assessed and showed that we were able to quantify at least 5 cells per microgram of DNA. This method was used in 38 blood DNA positive samples of 31 t(14;18)-positive FL patients in different remission status. Before treatment, 3 samples from 3 patients with different stages were analysed. The amount of cells quantified was as follows: less than 30 cells per mliter (c/ml) of blood for the stage I patient, 35 c/ml for stage III and 1800 c/ml for stage IV. During treatments, 9 blood samples (8 patients) were quantified. Three out of 9 were in complete remission (CR) or close to CR: few cells were detected. For the 5 other patients (6 samples), no CF was observed: we detected 27 to more than 80000 c/ml (median: 90 c/ml). Among the 19 remaining patients in CR, 3 groups could be distinguished: 1. out of 10 patients with less than 50 c/ml revealed (1, 20, 22 and 36 months after sample collection (ASC). Six of them remained disease free 12 to 36 months ASC (median: 31 months); 2. among 6 patients with 50 to 500 c/ml experienced relapse (1 and 8 months ASC), the 4 remaining patients being in CR 11, 20, 26 and 42 months ASC; 3. patients (8 samples) had more than 500 c/ml (range 840 to more than 80000). Two relapsed (6 and 3 months after first collection) and the last one was lost to follow-up just after collection.

We conclude that (1) competitive PCR is a suitable method for quantification of circulating t(14;18)-carrying cells in peripheral blood of patients with FL; (2) the reliability and rapidity of the method make it suitable for analysis of large series of patients thus compatible with the possibility to get statistically acceptable conclusions concerning prediction of relapse. Further efforts are to be pursued to be able to conclude about the clinical value of this quantitative approach.

MORPHOLOGIC TRANSFORMATION OF FOLLICULAR LYMPHOMA (FL) IS ASSOCIATED WITH SOMATIC MUTATION OF THE TRANSLATED BCL-2 GENE

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Ninety percent of low-grade FL carry the t(14;18) translocation. This event juxtaposes the bcl-2 oncogene to the immunoglobulin gene and leads to bcl-2 gene overexpression. Morphologic transformation of FL to high-grade lymphoma is associated with multiple secondary chromosomal abnormalities of the neoplastic cells. To analyze whether additional structural alterations of the translated bcl-2 gene are associated with morphologic transformation of FL, we PCR amplified, cloned and sequenced the major breakpoints region (MBR) and the open reading frames (ORF) of the translated bcl-2 oncogene in six paired samples of FL and subsequent diffuse large cell lymphoma (DLCL). In five cases, FL and DLCL cells were clonally related, as suggested by the identical MBR sequences, but in one case they were different. PCR single-strand conformation polymorphism (SSCP) and sequence analyses were performed for identification of structural alteration of the bcl-2 gene in the MBR region corresponding to the 239 amino acid p26-bcl-2a protein. In three of the six patients, a total of 11 point-mutations of the ORF were detected in the DLCL cells. Four of them, at positions 29, 46, 59 and 106, yielded amino acid replacements. These findings demonstrate that FL and DLCL cells may be clonally related or unrelated. They also show that transformation of FL cells can be associated with somatic point-mutations of the bcl-2 proto-oncogene ORF sequence resulting in alteration of the p26-bcl-2a gene product.

2. Biology
Single Specific Primer Amplification for Sequence Analysis of Clone Specific Variable Regions


Clonal antigen receptor (AGR) gene rearrangements are excellent markers for individual lymphoid clones and thus suitable for monitoring the occurrence of residual malignant clones during the treatment of leukemia and lymphoma.

Sequence information comprising the variable region of the respective rearranged AGR genes allows the design of clone specific primers that provide a highly sensitive and specific tool for the detection of residual clonal lymphoid cells.

Currently the clone specific variable regions are usually amplified using pairs of consensus primers. For that purpose an array of consensus primers must be assayed in order not to miss any clonal rearrangements. In contrast a single generally applicable protocol might be used if the known constant sequences at only one adjacent side of the variable region were considered.

Therefore two methods of ligation mediated single specific primer PCR were assessed. For anchor PCR an arbitrary sequence was ligated to cohesive ends, resulting from restriction digest of genomic or cDNA. This provided a primer binding sequence at the unknown end of the region to be amplified. In ‘passhandle’ PCR an anchor linker complementary to a part of the constant region was applied. After ligation and heat denaturation a hairpin structure formed, and nested primers binding to the stem region of the secondary structure allowed a highly specific amplification of the unknown variable loop sequence. The techniques of passhandle and anchor PCR were applied to constant and variable sequences of DNA obtained from the cell line Jurkat and yielded sequencable PCR products comprising the expected intron sequences or the variable part of the coding sequences of the T-cell receptor gene, as predicted from available sequence data.

Comparative genomic hybridization (CGH) detects many genetic abnormalities in follicular lymphoma. A. Vélot-Loiseau, H. Vigier M, Mellerin MP, Moreau A, Gaillard F, Hautoineau A, Batlle R et al. Laboratoire de HématoLogie, Laboratoire d'Anatomie Pathologique et Service d'Hématologie Clinique, Hotel-Dieu, Place Alexis Ricordeau, 44055 Nantes Cédex 01, France

To evaluate the usefulness of CGH in the analysis of follicular lymphoma, we studied 28 patients. This technique is based upon a competitive hybridization of equal amounts of tumor and normal DNAs, differentially labeled on normal human chromosomes. Then, by fluorescence analysis, the relative quantities of each DNA hybridized on these chromosomes can be evaluated, and a genomic map of gains and losses of tumor material can be determined. This technique offers the major advantage of avoiding the need of metaphase observation. We analyzed 28 patients for whom we disposed of a frozen lymph node sample (already extracted for IgC rearrangements examination). Among these 28 patients, 19 presented an aneuploidy with this technique. The most frequent abnormality was a gain of chromosome 18 material (trisomy 18 or gain of 18q) in 12 patients. The other non-random abnormalities were trisomy 7 and trisomy 8, found in 5 patients (twice simultaneously). Four presented chromosome 6 abnormalities (three deletions, twice associated with a 6p gain, and a gain of 6q in one patient) and 4 patients presented a trisomy 12. Finally, a 10q deletion was found in 3 patients. Nine patients presented normal profiles, which can be interpreted in 2 ways. The first hypothesis is the absence of any numerical changes in the tumor. This hypothesis is quite likely since some published cases displayed a reciprocal (14;18) as the sole abnormality. The second hypothesis is the contamination of the sample with normal cells, masking the presence of possible abnormalities.

In conclusion, this preliminary study demonstrates that CGH in follicular lymphoma shows cases that should allow us to look at possible clinical or prognostic correlations.

FREQUENT DELETIONS OF 6q23-24 IN FOLLICULAR LYMPHOMAS DETECTED BY MEANS OF FLUORESCENCE IN SITU HYBRIDIZATION

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Deletions involving the long arm of chromosome 6 (del(6q)) are among the most frequent chromosome aberrations in malignant lymphomas. In case of follicular lymphomas, del(6q) often occurs as secondary change in addition to t(14;18) and is therefore suggested to contribute to the histological transformation and clinical progression. It is now generally assumed that at least one tumor suppressor gene is localized in 6q, which is inactivated not only in lymphomas but also in different other tumors. In order to better define the regions of 6q that are consistently lost we studied 22 follicular lymphomas both by chromosome analysis and by fluorescence in situ hybridization (FISH). 14 of these were low grade and 8 were high grade follicular lymphomas. Dual color FISH was performed with two YAC DNA probes hybridizing at 6q23-24 and with the centromeric probe for chromosome 6 as an internal control. In control studies, the cut off limit for the detection of a deletion of 6q23-24 was defined at 5% interphase cells with one hybridization signal for 6q23-24, but two hybridization signals for the centromeric region. Deletions of 6q23 were found in 36% of the studied follicular lymphomas by chromosome analysis and in 64% by FISH. All cytogenetically detectable deletions were confirmed by FISH. Low grade follicular lymphomas showed del(6q) in 42% by chromosome analysis and in 72% by FISH; high grade lymphomas in 25% by chromosome analysis and in 50% by FISH. Lymphomas with a cytogenetically detectable del(6q) had a higher proportion of cells with del(6q) in FISH studies than lymphomas without a cytogenetically detectable del(6q). Del(6q) was detected in similar frequencies both in low grade and in high grade follicular lymphomas (39% compared to 45%). Moreover, del(6q) was seen in high grade follicular lymphomas both with and without t(14;18). These results indicate that 1) FISH analysis is more sensitive to detect del(6q) in follicular lymphomas than chromosome analysis or LOH studies and 2) the region 6q23-24 is more often deleted in low grade and in high grade follicular lymphomas with and without t(14;18) than suggested hitherto.

Inactivation of CDKN2A (p16INK4a) tumor suppressor gene (TSG) contributes to tumor progression in low grade lymphoid malignancies, S. K. Blandinger, J. W. Bardman, and O. I. Olopade Section of Hematology/Oncology, Department of Medicine, and Department of Pathology, The University of Chicago, Chicago, Illinois 60637.

The natural history of low-grade non-Hodgkin's lymphoma (NHL) is characterized by a prolonged indolent phase which is followed by progression to intermediate- or high grade lymphoma with a dismal prognosis. This clinical progression is often associated with detectable histologic changes but the genetic alterations involved in the transformation have not been well characterized. Recently, others have identified a tumor suppressor locus on the short arm of chromosome 9 harboring a cluster of genes including MTAP, CDKN2A and CDKN2B which is deleted in about 30% of Acute lymphoblastic leukemia (ALL) and more than 80% of lymphoid-derived tumor cell lines. (Olopade et al. PNAS 92:6449-6453, 1995). CDKN2A has been identified as the TSG in this locus because it encodes a 16kd protein (p16INK4a) which acts as a negative regulator of the cell cycle through its interactions with RB and CDK4/CDK6 proteins. To determine whether CDKN2A is involved in the transformation of diffuse large cell lymphoma (DCL), we have examined 11 cases of DCL; we examined 11 cases of DCL which evolved from low grade NHL, (transformed DCL); and 9 low grade NHL which had subpopulations of large cells with diffuse growth pattern (7 follicular NHL, 1 CLL, 1 mycosis fungoides). Interphase fluorescence in situ hybridization was performed on these samples using a 25kb cosmid contig (COSp15) encompassing CDKN2A. One of 11 cases of DCL (9%) and 1 of 9 low grade NHL (11%) had homologous deletions of COSp16. In contrast, all five transformed DCL (100%) had COSp16 deletions. Two cases had homologous COSp16 deletions, two cases had homologous deletions, and one case had a partial homologous deletion of the same cosmid. Thus, CDKN2A is frequently deleted in transformed DCL in contrast to de novo DCL (p < 0.01, Fisher's exact test) or low grade NHL. (p < 0.03). In addition to its critical role in ALL, these results suggest that CDKN2A deletion is a genetic marker for the histologic transformation from low grade to diffuse large cell lymphoma. Further studies will examine how this pathway can be manipulated to develop more effective and selective chemotherapy approaches in patients with ALL and transformed lymphoma.
A NOVEL TUMOR SUPPRESSOR GENE MAY RESIDE BETWEEN D13S5273 AND D13S5272 IN B-CELL
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In B-cell chronic lymphocytic leukemia (B-CLL) abnormalities of the long arm of chromosome 13, in particular of band 13q14, are the most common structural aberrations. Since the retinoblastoma tumor suppressor gene (RB-1) maps to 13q14 it was speculated that RB-1 is involved in the pathogenesis of the disease. Recently, the anonymous marker D13S253 which maps in close proximity to RB-1 was found to be deleted with higher frequency than RB-1 in B-CLL, and a novel tumor suppressor gene was proposed to be present at this locus. In this study we isolated cosmid clones containing D13S253 sequences and used them as a probe to analyze 131 B-CLL cases by FISH with RB-1 and c13S253 as probes: of 92 cases with two RB-1 copies, 27 exhibited hemizygous (n=18) or homozygous (n=9) deletion of D13S253. Of 38 cases with hemizygous deletion of RB-1, all but two cases also showed loss of D13S253 (hemizygous, n=32; homozygous, n=6). One case had a homozygous deletion of both loci. We conclude that deletion of D13S253 occurs in a substantial number of B-CLL without deletion of RB-1. However, in some cases there is a deletion of RB-1 without loss of D13S253, suggesting that the critical segment deleted in all cases maps to the genomic region between RB-1 and D13S253. To test this hypothesis we isolated cosmid clones containing a number of D-markers between RB-1 and D13S253. The most likely order of these markers in (centro-) D13S518 - RB-1 - D13S365 - D13S253 - D13S252 - D13S351 (tel.) was found. We used the cosmids for FISH on 22 B-CLL tumors with a loss of either RB-1 or c13S253: Among 20 tumors with a loss of RB-1 and two copies of RB-1 the centromeric border of the minimally deleted segment was between D13S252 and D13S272. In 2 tumors with a loss of RB-1 that retained two copies of D13S253 the telomeric border of the minimally deleted segment mapped between D13S273 and D13S272. From these results we conclude that a tumor suppressor locus of potential pathogenic significance in B-CLL may map between D13S273 and D13S272.

MOLECULAR CYTOGENETIC ANALYSIS OF HIGH GRADE NON-HODGKIN’S LYMPHOMA: A PILOT STUDY WITHIN A CLINICAL MULTICENTER TRIAL
Cytogenetic analysis of Non-Hodgkin’s Lymphomas (NHLs) within clinical multicenter trials has been hampered by the limited availability of fresh tissue samples. Thus only few data regarding the clinical implications of specific chromosomal aberrations are based on the prospective analysis of a homogeneous group of patients. The novel molecular cytogenetic technique of comparative genomic hybridization (CGH) allows a comprehensive analysis of chromosomal gains and losses using DNA obtained from paraffin embedded tissue samples. In order to evaluate the applicability of this method within a clinical multicenter trial, we performed a pilot study investigating high grade lymphomas. Paraffin material from 30 randomly selected patients was obtained from the pathological review laboratory of a German high grade lymphoma study group. CGH analysis was possible in 24 of 30 cases (80%). The frequent aberrations were gains on chromosomes 7 (8 cases), 1q (6 cases), 1q (6 cases), 1q (5 cases) and losses on the long arm of chromosome 6 (6 cases). In 3 chromosomal regions, high level DNA amplifications were found (mapping to chromosome bands 7q31, 1q13-14 and 1q22-23). These data show that (i) gains amplifications are not rare events in high grade lymphomas; (ii) chromosomal gains and losses are frequent in this group of malignancies (average of 3.5 changes per case); and (iii) there is a clustering of aberrations to a few chromosomal regions. Thus CGH may become an essential tool for the diagnosis of chromosomal gains and losses in clinical trials investigating NHLs. By allowing a more accurate correlation of molecular cytogenetic findings with morphological and clinical data, this technique may contribute to the refinement of lymphoma classification.

9p GENE LOSS IN POST TRANSPLANT LYMPHOMAS
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Investigation of growth regulation in low grade - B - cell Non - Hodgkin’s lymphomas is of pivotal importance for the development of new therapies. Recent data have underlined the central role of the CD40 activation pathway and the intramembrane conformation for B - cell stimulation. Aim of our in vitro study was to investigate the effect of different CD40 mAb (EA - 5, m4A9, the soluble human recombinant CD40 ligand gp9, BL - 4, a human monoclonal, and murine fibroblasts (the C32) of transfected fibroblast cell line LTK+ expressing CD32 on cell proliferation, apoptosis and in vivo growth of malignant B - cells in low - grade NHL. Peripheral blood or bone marrow samples from 17 patients with untreated B - CLL, 8 patients with follicular lymphomas and three patients with immunodeficiency were analyzed. The - CD40 mAb EA - 5 and m4A9 or the CD40 ligand gp9 alone did not stimulate the malignant B cells. BL - 4 enhanced cell survival of B - cells with a maximal 1,766 increase of cell viability on day 3 compared to the control cultured in medium alone (p = 0,001) and reduced apoptosis in 5/7 samples by a mean of 55% (p = 0,001). In a dose dependent and immunodeficiency lymphomas 56% (23 - 185) of the initial cell number survival on LTK+ cells versus 24% (0 - 67) in the control on day 6 (p = 0,001). Apoptosis was efficiently blocked by the mouse (foder cells from 35% (13 - 29) in the control to 5,5% (4 - 16) of B - CLL, from 55% (44 - 51) in the control to 7% (15 - 20) in follicular and immunodeficiency lymphomas. Reduction of apoptosis correlated with - cell - expression, which was significantly enhanced by the LTK+ cells. In contrast to induction of - phase - arrest could be observed. The stimulative effect of the LTK+ cells could not be substituted by C8b fibroblasts. BL - 4 significantly enhanced cell survival of B - CLL cells on LTK+ cells with a maximal 1,366 increase in cell number on day 7 compared to culture on LTK alone (p < 0,001) and an upregulation of the BL - 2 expression. Our data emphasize the decisive role of CD32 transfected fibroblasts and BL - 4 for survival of malignant B cells in low grade NHL in vivo. Cell survival is caused by an efficient reduction of apoptosis, accompanied by an upregulation of the BL - 2 expression.
Expression of the TCL1 gene in Hematologic Disorders

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TCL1 is a recently cloned gene located on chromosome 1q43.2. Little is known about the role of TCL1 in human neoplasia. We investigated 31 patients with leukemia: 60 Non-Hodgkin's lymphoma, 19 Hodgkin's disease, 5 carcinoma and 13 reactive lesions by means of Northern-blot and RT-PCR for the expression of TCL1 gene. No expression was found in carcinoma or myeloid leukemia whereas TCL1 was expressed in lymphoid neoplasms and reactive lymphoid tissue (11/11 PLL, 2/2 B-CLL, 7/9 ALL, 17/22 NHL of low grade, 12/19 NHL of high-grade malignancy, and 4/19 HD). We found that TCL1 expression is linked to the lymphoid system and that it is found frequently in NHLs and reactive lymphoid tissue. It is expressed in rare cases of Hodgkin's disease. Our results suggest that TCL1 may play a role in lymphomasclerosis. TCL1 is not restricted to a particular type of Non-Hodgkin lymphoma.

PROLIFERATION OF MANTLE CELL LYMPHOMA AND CHRONIC LYMPHO-CYTIC LEUKEMIA BY ACTIVATION OF THE CD40 AND CD72 ANTIGEN

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Although mantle cell lymphoma (MCL) and CLL have clear morphological and immunologic (CD5 expression) similarities, and they are both derived from the mantle zones of follicles, their clinical behavior is quite different. In contrast to CLL, the clinical course of MCL is aggressive and characterized by therapy-resistance. Here, the proliferative capacity of CLL and MCL cells is examined in vitro, using the CD40 system and anti-CD72 monoclonal antibodies (mAbs). Briefly, in the system the CD40 antigen, a pan-B cell antigen, is crosslinked by anti-CD40 mAbs using a human FcyRI receptor-expressing mouse fibroblast cell line (C32L cell). CD72 is also a pan-B cell antigen, and is the ligand for CD40. CD40 is expressed on T cells, a small subgroup of normal B cells (80%), and on CLL and MCL. It is unknown, if reciprocal interactions between CD72 and CD20 occur. Here, we stimulated the CD72 antigens on malignant B cells with agonistic anti-CD72 mAbs.

Methods: T cell depleted (5CD3 < 0.5) malignant B cells from patients with CLL (n=11), and MCL (n=7) were cultured (1x10^7/100 μl) in 6-fold for 7 days in 96-flat bottom culture plates with irradiated CD32L cells (5x10^5/100μl) and anti-CD40 mAb 89 (0.5μg/ml) in the presence of IL-4 (50 ng/ml). Besides, CLL and MCL cells were incubated with the anti-CD20 mAb WL 225 (1 μg/ml). Proliferation was assessed by [3H]-thymidine incorporation and cell counting.

Results: Cultures from 11 CLL cases showed a significantly increased in activation and proliferation (2-4 fold) of cells after CD40 cross-linking, whereas in 5 of 7 cases of MCL no proliferation could be induced, although these cells express the CD40 antigen. In 4 out of 8 CLL cases, and 1 out of 4 MCL cases proliferation was seen (3-5 fold) increased in [3H]-thymidine incorporation upon CD72 activation. In 3 out of 5 CLL cases tested, and in 1 out of 3 MCL cases tested, crosslinked anti-CD72 mAbs exerted a cytostatic effect. The phenotype of the cells remained unchanged after culture. In two cases tested, cytotoxic analysis of the CLL cells showed before and after culture a shift, del(11q) abnormalities, and an inst(11q)(1q32:15q12).

Conclusion: The proliferative capacity of mantle cell lymphoma and CLL cells in vitro is completely different, and warrants further investigation.

T-CELL DERIVED CYTKINES COSTIMULATE PROLIFERATION OF CD40-ACTIVATED GERMINAL CENTER B-CELLS AS WELL AS FOLLICULAR LYMPHOMA CELLS

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Follicular lymphomas are the malignant counterparts of normal germinal center B-cells. These neoplasms grow in vivo in close association with substantial numbers of polyclonal T-cells, predominantly from the T-helper cell type. T-cell derived growth factors are known to be involved in the development of germinal center cells. However, their role in the pathogenesis of follicular lymphomas remains unclear. It has recently been shown that sustained proliferation of germinal center and follicular lymphoma cells can be induced through the CD40 pathway. Using this in vitro culture system, we investigated the costimulator activity of 14 different cytokines on the proliferation of CD40-activated follicular lymphoma cells in comparison to germinal center B-cells. CD19+ cells were isolated from tonsils (n=3) or diagnostic lymph node biopsies from patients with follicular (n=5) or transformed (n=3) lymphomas, and grown on irradiated CD40+transfected mouse fibroblasts. Proliferation was determined by measuring [3H]-thymidine incorporation after 7 days of culture in the presence of recombinant cytokines, IL-4, and to a lesser extent, IL-10 and IL-13, proved to be the most potent and consistent costimulators of proliferation for both germinal center B-cells and follicular lymphoma cells. Therefore, the data presented here are T-cell derived cytokines suggests that lymphoma infiltrating T-cells play a role in the growth of these malignancies. Moreover, the finding that proliferation of follicular lymphoma and germinal center cells are costimulated by the same cytokines indicates that the responsiveness to paracrine growth factors is not a characteristic of the malignant phenotype.

IMMUNOPHENOTYPIC AND MOLECULAR ANALYSIS IN LYMPHO-PROLIFERATIVE DISEASES

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We performed an immunocytochemical and molecular analysis of 413 pathologic samples (347 peripheral blood, 67 lymph node biopsies, 98 diseases, admitted to our Institution during the last five years. The samples of PB were obtained from patients with persistent lymphocytosis (5x10^7/l) and absence of. We performed an immunocytochemical and molecular analysis of 413 pathologic samples (347 peripheral blood, 67 lymph node biopsies, 98 diseases, admitted to our Institution during the last four years. The samples of PB were obtained from patients with persistent lymphocytosis (5x10^7/l) and absence of lymphopenia. The main diagnoses and the corresponding clinical findings are listed in Table 1.

In conclusion, the results of this study confirm the usefulness of immunocytochemical and molecular analysis in the diagnosis and classification of lymphoproliferative disorders.
A NOVEL MODEL SYSTEM FOR THE STUDY OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS (KSHV).

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The recently identified Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) has been found to be consistently associated with an unusual subset of AIDS-related lymphomas, the so-called body-cavity based lymphomas (BCBL). These lymphomas are characterized by a unique spectrum of morphologic and molecular characteristics, and grow as lymphomatous effusions without an identifiable contiguous tumor mass. Until now, efforts to delineate the role of KSHV in the pathogenesis of BCBL have been hampered by the lack of appropriate model systems and the concomitant presence of Epstein-Barr Virus (EBV) in most cases examined, and in all previously established cell lines. We now report the establishment and characterization of a novel BCBL cell line, BC-3, which is shown by PCR to be KSHV-positive yet EBV-negative as assessed by PCR for EBER, EBNA-2 and EBNA-3C. This cell line was established from a lymphomatous effusion obtained from an HIV-negative patient. BC3 cells express an indeterminate immunophenotype, with presence of CD45 but absence of B- and T-cell associated antigens. However, Southern blotting analyses show that BC-3 cells have clonal Ig heavy-chain gene (JH) rearrangement and germline TCR-α chain, thus confirming their B-cell origin; BC-3 cells also lack c-myc proto-oncogene rearrangements. These immunophenotypic and molecular features are consistent with the diagnosis of FBCBL. The cells both harbor and actively produce KSHV particles as judged by the presence of KSHV DNA which is resistant to DNase digestion, as well as by pulsed-field gel electrophoresis and electron microscopy studies. Consequently, the BC-3 cell line represents a unique and invaluable tool as a source of KSHV, for both the evaluation of the pathogenic potential of this virus and the mechanistic characterization of its role in the development of Kaposi's sarcoma and malignant lymphoma.

A NOVEL 70KD ENDONUCLEASE IN TdT-POSITIVE CELLS
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TdT is a 61Kd DNA polymerase uniquely expressed in normal pre-B and pre-T lymphocytes, and their neoplastic counterparts in leukemia and lymphoma. It is also the addition of deoxyriboonucleotides to the 3'-OH ends of DNA in a template-independent manner. TdT is also known to have a minor endonuclease activity. While the polymerase function of TdT has been associated with N-terminal inactivating, and no physiological function has yet been ascribed to TdT as an endonuclease. We now suggest that the endonuclease activity of TdT may be responsible for generating the 180-bp DNA fragments seen in TdT-positive cells undergoing apoptotic cell death following exposure to cytotoxic agents. We induced apoptosis (defined by membrane blebbing; volume loss; chromatin clumping; and DNA digestion into 180-bp monomers) in TdT-positive cell lines (Nalm-6; CEM; Molt-4) within 6-48 hours of incubation with single-agent dexamethasone, Ara-A, F-ara-AMP, or double-agent corticosteroid/dexcomycin. To detect endonuclease activity whole cell lysates were electrophoresed in DNA-loaded polyacrylamide gels, reconstituted in nucleic buffer and stained with ethidium bromide. Nuclease activity was defined by negative ethidium bromide staining. Processing whole cell lysates from TdT-positive cells in this manner revealed a previously undescribed 70Kd nuclease activity which increased 5-10 fold in 6-24 hours following drug exposure. TdT-negative cells (HUT-102; K562; HL-60) had no such 70KD endonuclease activity. To demonstrate apoptotic DNA digestion by the 70KD nuclease, a 70KD fraction from TdT-positive whole cell lysates, was incubated for 1 hour with HelA target nuclei. Electrophoresed on agarose gels revealed apoptotic 180-bp digestion of HelA DNA by the 70KD material in the presence of Ca++ and Mg++. Western Blot staining of whole cell lysate gels from TdT-positive cells confirmed the presence of TdT in these nuclease bands. Work in progress is directed at further characterization of one of the 70KD apoptotic nuclease.

EPSTEIN-BARR VIRUS LATENT GENE EXPRESSION IN KAPOSI SARCOMA-ASSOCIATED HERPESVIRUS - CONTAINING BODY CAVITY-BASED LYMPHOMAS
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Body cavity-based lymphomas (BCBCL) are an infrequently occurring subset of non-Hodgkin lymphomas (NHLs) that are uniformly infected with Kaposi sarcoma associated herpesvirus (KSHV), BCBCLs are mostly AIDS-related but also occur rarely in elderly HIV-negative patients. They grow exclusively or mainly in the body cavities as lymphomatous effusions, nearly always in the absence of an identifiable contiguous tumor mass. BCBCLs exhibit immunohistoclastic plasmacytoid morphology, an indeterminate immunophenotype, clonal immunoglobulin gene rearrangements and clonal Epstein-Barr virus (EBV) infection in the absence of c-myc oncogene rearrangements. We analyzed EBV latent gene expression at the mRNA level to determine the pathogenic role of this agent in BCBCL. We selected five BCBCLs coinfected with EBV and KSHV, and performed reverse transcription-PCR for a panel of EBV latent genes. EBER-1 mRNA, a consistent marker of viral latency, was positive in all cases, although at lower levels than in the non-BCBL controls. With Cp-initiated mRNAs, encoding all EBNA and characteristic of latency 3, were negative in all cases. Sp-initiated mRNA, encoding only EBNA-1 and characteristic of latencies 1 (L1) and 2 (L2), was positive in all cases. LMP-1 mRNA expression in latencies 2 and 3 (L3), was present in all cases. BZF-1, an early lytic phase marker, was weakly strongly positive, demonstrating a variable degree of productive viral infection. Therefore, BCBCLs exhibit a latency 2 pattern similar to that of nasopharyngeal carcinomas and Hodgkin disease. This situation is different from other B-cell neoplasms such as Burkitt lymphoma (L1), post-transplantation lymphoproliferative disorders (L3) and EBV positive AIDS-related immunoblastic lymphomas (L3). This restricted expression of EBNA-1 and LMP-1 suggests that EBV may not be fully responsible for malignant transformation in these BCBCLs, strengthening the notion that KSHV plays a crucial role in their pathogenesis.

IS IP-10, A CYTOKINE CHEMOTACTIC FOR CD4+LYMPHOCYTES, A FACTOR IN THE EPIDERMOTRYPISM OF CUTANEOUS T-CELL LYMPHOMAT?
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Human interferon-γ-inducible protein 10 (IP-10), is a C-X-C chemokine that is chemotactic for CD4+ lymphocytes in vivo and in vitro and inhibits the growth of immature normal and leukemic hemopoietic progenitors and of T-cell clones. The IP-10 receptor is constitutively expressed on T-cell malignancies (CTCL). IP-10 is an inducer of apoptosis and proliferation of CTCL blasts. We investigated the expression of IP-10 in normal skin and lesional CTCL biopsies by immunohistochemistry. We generated polyclonal rabbit antisera by injections of highly purified IP-10 or recombinant fusion proteins containing all 10 IP-10 sequences. The sera recognized and neutralized IP-10 but not any other keratinocyte proteins. With immunoperoxidase staining we demonstrated that IP-10 was expressed predominantly in basal epidermal keratinocytes of normal skin. In CTCL lesions, where the epidermis overlying the malignant lymphocytes was hyperplastic, IP-10 expression was increased and extended to the suprabasal keratinocytes in 20 of 21 patients, but was detected in the malignant CTCL lymphocytes in only three of them. Skin clinically free of CTCL demonstrated normal IP-10 immunostaining. In patients who had matching biopsies performed before and after treatment, IP-10 was overexpressed before treatment, but was normally expressed in the post-treatment biopsy that demonstrated resolution of the CTCL. Epidermal hyperplasia or increased IP-10 immunoreactivity were not seen in any of four patients with B-cell lymphoma involving the dermis. Primary normal human keratinocytes cultured in vitro with purified recombinant IP-10 exhibited a dose-dependent increase in DNA synthesis that was abolished by the addition of the medium of antibodies neutralizing IP-10. These data suggest that CTCL epidermal keratinocytes secrete increased amounts of IP-10, that could attract the malignant lymphocytes to the skin and contribute to the epidermal hyperplasia of CTCL. Further work is needed to determine the role of IP-10 in the growth of CTCL with the aim of providing curative therapies.

2. Biology
GENE TRANSFER OF COSTIMULATORY MOLECULES BY RECOMBINANT ADENO-ASSOCIATED VIRUS (AAV) VECTORS ENHANCES THE CYTOLYTIC T-LYMPHOCYTE (CTL) RESPONSE TO MULTIPLE MYELOMA (MM) CELLS IN VIVO

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It was shown that costimulatory molecules of the B7 family, i.e. B7-1 and B7-2, can be utilized to induce a potent anti-tumor immune response and protection in different rodent tumor models. MM cells which partly lose expression of B7 antigens are a potential target for this approach. The human B7-1 and B7-2 genes were efficiently packaged into high titer rAAV with an improved system, and MM cell lines (LP-1, RPMI 8226) were transduced by purified rAAV particles. Proliferation of prestimulated human allogeneic T cells in response to rAAV/B7-1 and rAAV/B7-2 transduced, r-irradiated MM cells was assessed by [3H] thymidine incorporation, by RT-PCR based detection of immunomostulatory cytokine transcripts, and by ELISA quantification of cytokines in the supernatant. Stimulation of T cells with rAAV/B7-1 or rAAV/B7-2 transduced MM cells resulted in a 2 to 10 fold higher proliferative T cell response in comparison to tumor cells transduced with AAV vectors coding for control genes. The stimulatory effects of B7-1 and B7-2 were of equal potency and correlated with an enhanced secretion of IL-2 and IFN-γ by stimulated T cells. Furthermore, [3H] thymidine release assays of MM cells showed that the CTL response was stronger in response to B7-1 or B7-2 transfected cells than to controls. Both CD4+ and CD8+ T cells were present after repeated stimulation with rAAV/B7-1 or rAAV/B7-2 transduced MM cells, suggesting that both populations might contribute to the cytolytic response. Taken together, these experiments show that rAAV-mediated transfer of B7 genes is able to increase the CTL response to MM cells.

mRNA expression of genes involved in classical and in apoptosis associated drug resistance (MDR1, MRP, GSTx, Topoisomerase I, IIa, bcl-2, fas, bcl-x and BAX) in patients with Non Hodgkin Lymphomas (NHL).

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We used a modified quantitative reverse transcriptase PCR assay (Van Hille B, Lotri A et al, Clin Chem 6/95) to assess the messages from genes involved in drug resistance (DR) related to transmembrane proteins (MDR1, MRP, MDK associated protein gene), intracellular drug detoxification (Glutathione S Transferase isofoms γ [GSTx]), cell proliferation (TopoisomeraseTopo II) and also genes associated to apoptosis related DR bcl-2, fas, bcl-x. The results are expressed in a relative ratio of the gene of interest versus β-actin, which was used as a housekeeping gene. We evaluated 49 biopsies from patients with Non Hodgkin's lymphomas (Low Grade n=18, High Grade n=21, paratidic n=4, relapsing/transformed, n=6). Results: MDR1, MRP and GSTx mRNA levels were not significantly associated with any morphological or clinical characteristic. Topo II mRNA levels were associated with the Ki-67 proliferation marker measured by immuno-histochemistry (p<0.001; r=0.76) and, consequently, Topo II was lower in patients with Low compared to High Grade lymphomas (p<0.02). Bcl-2 mRNA tended to be lower and Fas mRNA expression higher in T-cells, compared to B-cell lymphomas. No associations with the International Prognostic Index (IPI) were obvious. Work on the BAX gene is ongoing. Conclusions: The expression of some of the gene messages tested seems to be linked to certain lymphoma entities and no correlations to the IPI or clinical outcome are obvious. This latter question needs to be addressed in larger groups of patients (supported by the Swiss National Foundation).

INTERLEUKIN-15 INHIBITS PROLIFERATION OF CHILDHOOD BURKITT'S LYMPHOMA CELLS

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Interleukin-15 is a newly discovered T-cell growth factor which resembles interleukin-2 in its tertiary structure and in many of its biological functions. To examine whether IL-15 is involved in the pathogenesis of pediatric Burkitt's lymphoma, we investigated the expression and regulation of the IL-15 cytokine itself, of the two signal chains of the IL-15 receptor and the proliferative response to IL-15 in a panel of 14 Burkitt's lymphoma cell lines. To study inducibility of IL-15, cells were stimulated by IL-7, PMA, TNF-α, or calcium ionophore A23187. IL-15 cytokine expression and expression of the IL-2/IL-15 beta and common gamma-chain of the receptor was studied by reverse-transcriptase PCR followed by Southern hybridization with a labeled internal oligonucleotide. Expression of the IL-2/IL-15 receptor beta-chain was also studied by flow cytometry (FCM). Proliferation of cells in the presence of IL-15 was studied by MTS assays. IL-15 was highly expressed in 3 of 14 cell lines and to a lower extent in 3 further cell lines. Some negative cells could be induced to express IL-15 upon stimulation with PMA or A23187, but not with IL-7. IL-2 expression was tested as a control and was found to be negative in all cell lines. The IL-2/IL-15 receptor common gamma-chain was detected in all Burkitt’s lymphoma cell lines. The beta chain showed varying expression by PCR and by FACS analysis in 6 out of 14 cell lines. The IL-2 receptor alpha-chain was used as a control and was found to be expressed in all except one of the cell lines when tested by RT-PCR. IL-15 inhibited growth in 2 of 13 cell lines, but all other cells were unaffected. We conclude, that IL-15 and the two known chains of its receptor can be expressed in childhood Burkitt’s lymphoma cell lines. IL-15 may be an autocrine growth inhibitory factor for some Burkitt’s lymphoma cell lines.

SOLUBLE CD30 DECREASES THE AVAILABILITY OF CD30 LIGAND (CD30L) ON PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH CD30+ TUMORS. A MECHANISM FOR EVADING IMMUNOSURVEILLANCE?


CD30L is a member of the TNF ligand family that can induce cell death of several CD30+ cell lines. It is expressed on resting B cells as well as activated T cells of normal donors. However, resting peripheral blood B cells of patients with CD30+ tumors (Hodgkin's disease and Ki-1+ lymphoma) had lower expression of CD30L compared to normal donors (57.6%+6% vs. 80.6%±2.7%) (Younes et al; Proc ASH 1995). These B cells can upregulate CD30L expression in response to PHA stimulation suggesting that this observed low expression may be due to extrinsic factors rather than primary B cell damage (Younes et al, Proc ASH 1995). In order to determine the role of soluble CD30 in the downregulation of CD30L in patients with Hodgkin's disease and Ki-1+ lymphoma, we measured CD30L expression on peripheral blood lymphocytes from 11 individuals and correlated them with plasma soluble CD30 levels as determined by ELISA. A linear reverse correlation between soluble CD30 and CD30L expression on B cells was found with an r=0.72. Furthermore, exogenous soluble CD30 (1,4, 10μM) decreased the detection of CD30L on PHA-induced normal peripheral blood lymphocytes. Because CD30L can induce cell death of CD30+ cells, the elevated serum levels of CD30 (which is frequently detected in patients with Hodgkin's disease and Ki-1+ lymphoma) may give the malignant CD30+ cells proliferative advantage by decreasing CD30L availability on the normal lymphocytes. Our finding may explain the previously reported observation that patients with Hodgkin's disease and Ki-1+ lymphoma who have elevated levels of soluble CD30 have poor prognosis.
HBV CARRIERS IN THE TREATMENT OF MALIGNANT LYMPHOMA: AN EPIDEMIOLOGICAL STUDY IN JAPAN.


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In the HBV endemic area, severe hepatitis associated with cancer chemotherapy is one of the serious complications. The prevalence of HBV carriers, the incidence and the risk factors of hepatitis in HBV carriers with malignant lymphoma were investigated by questionnaire to the LTSG members. Among 1,380 patients collected from 8 institutions through Japan, 45 (3.26%) pts. were determined to be HBV carriers (positive HBs - antigen). Hepatitis developed in 17 (37.8%) of 45 HBV carriers; 7 pts. terminated in fatal hepatic failure. The state of seroconversion and chemotherapy regimens were conspicuous among the risk factors: Hepatitis developed in 11 (61.1%) of 18 pts. treated with the 3rd - generation chemotherapy vs. 6 (28.6%) of 21 pts. with the 1st - generation chemotherapy; in 13 (50.0%) of 26 pts. with positive HBe - Ag and positive HBs - Ab (seroconverting) vs. 1 in (2.5%) of 8 pts. with positive HBe - Ab and negative HBs - Ab (non - seroconverted) vs. 1 (14.3%) of 7 pts. with undetermined serology. Hepatitis terminated fatal hepatic failure in 6 pts. treated with the 3rd - gene. chemotherapy vs. 1 pt. treated with the 1st - gene. chemotherapy; in 7 seroconverted pts. vs. no non - seroconverted pts.

Inhibition of HBV replication during chemotherapy by INF could prevent the development of severe hepatitis in HBV carriers.

APOPTOTIC CYSTEINE PROTEASES CPP32 AND ICH-1/LC3 DISPLAY INVERSE PROFILES OF EXPRESSION IN NON-HODGKIN'S LYMPHOMAS AND HODGKIN'S DISEASE.

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The family of ICE-related cysteine proteases includes ICE, Mch2, ICH-1/ICEd, ICH-2/IX, and CPP32/YAMA. These molecules act as effectors of apoptosis via proteolytic cleavage of poly(ADP-ribose) polymerase (PARP). Previous reports showed a preferential expression of CPP32 in lymphoid cell lines, which prompted us to search for a potential involvement of ICE-related proteases in the pathogenesis of malignant lymphomas. Monoclonal antibodies directed against CPP32 and its homolog ICH-1 were used for immunohistochemistry on paraffin sections. 54 samples representing various types of non-Hodgkin's lymphomas (NHL) and Hodgkin's disease (HD) were analyzed in parallel with a control group of 6 benign reactive lymph node and spleen.

In normal lymphoid tissues, expression of CPP32 was restricted to germinal center cells and a few activated cells scattered in the interfollicular area. ICH-1 was negative in germinal centers and occasionally positive in mantle zones. All follicular NHLs and most high grade NHLs were CPP32-positive. Reed Sterngberg cells (RSC) in HD were CPP32-negative and surrounded with a ring of CPP32-positive small lymphocytes. In contrast, ICH-1 was negative in most NHLs, except in T-cell rich B-cell NHLs where positivity was restricted to large neoplastic B-cells surrounded with ICH-1-negative small lymphocytes. RSC were often ICH-1-positive, and surrounded with ICH-1-negative reactive lymphocytes. It appears therefore that some HD and NHLs cases display inverse profiles of cysteine protease expression. These findings suggest that ICE-related enzymes, despite their shared catalytic properties, may differ in substrate specificities and have different functions in benign and neoplastic lymphoid tissues.

ICAM-1 (CD54) IN NON-HODGKIN'S LYMPHOMA (NHL): RELATIONSHIP WITH TUMORAL DISSEMINATION AND PROGNOSTIC VALUE.

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The immunoglobulin adhesion molecule ICAM-1 has been recently correlated with both mechanisms of lymphoma dissemination and the prognosis of patients with NHL. The tumoral expression of ICAM-1 (CD54-10L54) was studied in a series of 58 patients (33M/25F, median age, 58 years) diagnosed with NHL between 1987 and 1990 at a single institution. A streptavidin-biotin alkaline phosphatase method was used for the analysis, evaluating the ICAM-1 expression in a semiquantitative manner: negative (-), weak (+), positive (++), and strongly positive (+++). The histological distribution of the cases was the following: Small lymphocytic (S), follicular (F), mantle-cell (M), diffuse large-cell (DL), and lymphoblastic (L). Four (7%) patients were in stage I, 10 (17%) in stage II, 10 (17%) in stage III, and 34 (59%) in stage IV. Biliary disease was observed in 21 pts (36%), and extranodal involvement in 41 (71%) (bone marrow in 24 cases). All the patients received Adriamycin-containing regimens as first line therapy, 24 pts (41.4%) achieving a complete remission (CR). ICAM-1 expression was negative in 10 cases (17%), weak in 22 (38%) and strongly positive in 9 (15%). No significant relationship between ICAM-1 expression and histological subtype was found, of note, however, all the patients with mantle-cell NHL were ICAM-1 (+) or (+). Patients with negative or weak ICAM-1 expression have more frequently disseminated (stage IV) disease (77 vs. 35%, p<0.004), extranodal involvement (87 vs. 52%, p=0.008) and bone marrow infiltration (61 vs. 19%, p=0.005) than the others. Positive ICAM-1 pts achieved more frequently CR (53 vs. 22%, p=0.04), and their survival was significantly better than in the ICAM-1 negative group (2-year survival 79 vs. 17%, respectively, p<0.001). When the group of intermediate grade NHL was analyzed, ICAM-1 expressions also correlated with stage, extranodal involvement, and bone marrow infiltration (p=0.008), CR achievement (70 vs. 35%, p=0.057), and survival (2-year survival 70 vs. 17% p=0.008). These results confirm the importance of ICAM-1 (CD54) in the dissemination of the disease and prognosis in NHL patients.

Prognostic value of T cell and S-phase fractions in B-cell non Hodgkin's lymphomas


In lymph nodes or extranodal biopsy specimens, in non Hodgkin's lymphomas (NHL) of B-cell type residual or reactive T-lymphocytes are found. In this investigation 71,34 high grade lymphomas (HGM) and 37 low grade lymphomas (LGM) consecutive and previously untreated adult patients were analyzed with respect to S-phase and T-cell fractions in bone marrow material by flow cytometry.

A significant correlation was found between high S-phase rate and low T-cell fraction (p=0.035). The same result was found among HGM (p=0.026) but not in LGM. No difference was found concerning the levels of T-cells in the two subgroups, HGM median 20 (range 3-64), LGM median 26 (range 5-65). A significant correlation between low T-cell fraction and high clinical stage was also found (p=0.034). In the group of LGM a border-line value was found (p=0.055), among HGM no correlation was found. Significant shorter survival times were found for patients with tumours showing a high fraction of S-phase cells (>4%) (p=0.002), this result remained true in the group of high grade (HGM) lymphomas (>7%) (p=0.031) but not in the low grade (LGM).

It was further noted a significant difference in survival, with a worse outcome for patients with a low T-cell fraction (<20%) (p=0.008), also this result remained true among HGM (p=0.030). A difference not reaching statistically significance was found among LGM (p=0.082). A multivariate analyses showed age (p=0.013), stage (p=0.018) and T-cell fraction (p=0.038) to be independent prognostic factors. We conclude T-cell fraction as an additive prognostic factor in malignant B-cell lymphomas, in this study superior to proliferation rate. Its prognostic value seems to be higher in HGM where a correlation to proliferation rate has been observed. In LGM T-cells were found to have less prognostic value than in HGM, no correlation to S-phase was observed. Instead a correlation to clinical stage was found, and the prognostic value in this group, could to same extent depend on this. As an explanation one could suggest a difference in interactions between the malignant B-cell alone and the reactive T-cells between the two morphological subgroups.

2. Biology
S PHASE FRACTION, BUT NOT APOPTOSIS PREDICTS PATIENT SURVIVAL IN MALIGNANT B-CELL LYMPHOMAS.

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A dual laser, triple colour flow cytometry method was developed to measure the fraction of apoptotic cells and cells in the S phase of the cell cycle in 95 cases of B-cell lymphomas. The data were gated on tumour characteristic light chain (κ or λ) expression. The mean fraction of apoptotic tumour cells and S phase tumour cells were 2.1% (SD=2.9%, median 1.1%) and 4.7% (SD=7.8%, median 1.4%), respectively. High grade lymphomas had a higher tumour specific S phase fraction (mean=10.7%, n=30) than the low grade ones (mean=1.9%, n=65, p<0.0001), but the tumour specific fractions of apoptotic cells were similar (mean 2.5% and 1.9% for high and low grade cases, respectively, p=0.58).

There was no significant correlation between proliferation and apoptosis (p=0.07, linear regression on log transformed data).

Treatment was given according to standard recommendations: Low grade lymphomas with a wait and see policy, chlorambucil/prednisone or COP (cyclophosphamide, vincristine, prednisone), high grade lymphomas with an adriamycin containing combination chemotherapy regimen. The median age of the patients was 55 years, the median observation time of the survivors 40.6 months. A high S phase fraction was a powerful independent predictor of survival (ten year estimated survival for the low S phase group 62%, for the high S phase group 7%, p<0.0001). The difference in survival was highly significant for the low grade lymphomas (p<0.0001) but not for the high grade lymphomas when analysed separately. S phase was a more powerful predictor of survival than those in the international prognostic index (IPG; age, stage, performance status, number of extranodal sites and LD) for the whole group of lymphomas and for the low grade ones. Survival was not associated with apoptosis, even when the high and low grade cases, or the cases with high and low S phase were analysed separately.

Low grade lymphomas with high S phase fraction should be included in randomized trials in which aggressive therapy (i.e. high dose therapy with stem cell support) is tested.

Eu-BCL1 Rransgenic mice develop a B-CELL EXPANSION, SPLENOMEGALY, AND LYMPH NODE ENLARGEMENT.

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BCL1 is a candidate proto-oncogene that was discovered by cloning the (14;19) breakpoints of human chronic lymphocytic leukemias. Leukemic cells from such patients show overexpression of BCL1. BCL1, an iκB protein, acts by regulating the NF-κB family of transcription factors. Its role in tumorigenesis is unclear, but may involve dysregulation of genes regulated by NF-κB that are important in cell proliferation, differentiation or survival. In order to investigate the above we have generated six lines of BCL1 transgenic mice. Human BCL1 expression was targeted to B-lymphocytes using a transgene containing the immunoglobulin heavy chain enhancer a and VJβ promoter. Expression of the transgene in each line was shown by RT-PCR using primers that spanned an intron within the construct. Transgenic mice appear to develop normally compared to non-transgenic litter mates. So far we have examined mice between the ages of 7 to 12 weeks from 5 of 6 lines; each mouse was compared to a non-transgenic litter mate. Gross examination revealed the presence of obvious mesenteric lymph node enlargement and splenomegaly in mice from 2 of the 5 lines examined. In those animals in which splenomegaly was not obvious, spleen weights were greater in transgenic compared to control animals in 4 of 4 pairs. Flow cytometric analysis was performed on single cell suspensions from spleen, lymph node and thymus from each line and compared to negative litter mates. The relative proportions of B- and T-cell subsets were compared using CD45R/B220 and Thy1 antibodies. In all cases, except for 1 pair, there was an increase in the percentage of B-cells in lymph node and thymus of the transgenic animals as compared to matched controls (#p=0.008, Wilcoxon signed ranks test). Using flow cytometric methods we have not detected a difference in apoptosis either spontaneously or in response to dexamethasone treatment. Taken together, our preliminary results suggest an important role for BCL1 in B-cell physiology, consistent with its involvement in B-cell neoplasia. Further characterization of these mice will be presented.

CHARACTERISTICS OF HUMAN EPSTEIN-BARR VIRUS (EBV)-SPECIFIC CYTOтокIC T LYMPHOCYTES UTILIZED FOR ADOPITIVE IMMUNOTHERAPY OF EBV-INDUCED HUMAN B-CELL LYMPHOPROLIFERATIONS IN XENOGRAFTED C.B-17 SCID/SCID MICE.

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We have shown that unirradiated human Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes (EBV-CTL) generated in vitro from EBV-seropositive donors administered i.p. or i.v. significantly prolong the survival of C.B-17 scid/scid mice bearing autologous but not HLA-mismatched i.p. or s.c. monoclonal EBV-induced B-cell lymphoproliferations (EBV-LPD). In the present study, we demonstrate that the HLA-restricted activity displayed by EBV-CTL both in vitro and in vivo correlates with their in vivo homing pattern, and characterize these putative EBV-specific and HLA-restricted effectors. EBV-CTL were CD23+, CD169+, TCR αβ-, HLA-DR+, predominantly CD57* (75.7-98.6%) and had a high CD25 expression (48.1-83.2%). These cells were also positive for CD11a/CD18, CD54, CD58, CD44, CD49d, CD208 and CD28, and negative for CD45RA, CD11b, CD11c, and CD80. After 26 days in culture, their strong cytotoxicity against the autologous EBV-transformed B-cell line (EBV-LCL) (E/T ratio 6:1 34 4-56.4%) was inhibited by the addition of anti-CD3 mAb (75-80% inhibition), and predominantly abrogated by anti-HLA class I mAb (50-80% inhibition) vs anti-HLA class II mAb (10-30% inhibition). These cells had a high EBV-specific CTL precursor frequency against autologous EBV-LCL (1/25 to 1/125). We also evaluated the proliferation (3H incorporation) of unirradiated and irradiated (1,000, 2,000, 3,000 rads) EBV-CTL after 2, 5 and 8 days (D) of culture with the autologous EBV-LCL, in the presence and absence of IL-2, as well as their cytotoxicity against the autologous EBV-LCL. Irradiated EBV-CTL and unirradiated EBV-CTL without IL-2 failed to proliferate after more than 2 days in culture (< 1,000 CPM), while unirradiated EBV-CTL with IL-2 formed large colonies and had 74 incorporation assays, the in vitro cytotoxicity of irradiated EBV-CTL was comparable to that of unirradiated EBV-CTL. However, it remains to be determined whether irradiated EBV-CTL are capable of homing to EBV-LPD in vivo and to mediate a therapeutic response, such as we have observed with unirradiated EBV-CTL.

METASTATIC POTENTIAL OF LYMPHOMA/LEUKEMIA CELL LINES IN SCID MICE IS CLOSELY RELATED TO EXPRESSION OF L-SELECTIN AND CD44.

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Most mature lymphocytes recirculate continuously between the blood and the lymphoid organs via high endothelial venules. This step is mediated by interactions between the lymphocyte homing receptors expressed on the lymphocyte cell surface and the endothelial ligands. Because non-Hodgkin's lymphomas represent the malignant counterparts of normal lymphocytes, adhesion molecules regulating normal lymphocyte traffic may also be involved in the dissemination of these malignancies. In this study, we have explored the correlation between the expression of 6 adhesion molecules (LFA-1, ICAM-1, CLA, VLA-4, L-selectin and CD44) and dissemination of the lymphoma/leukemia cells transplanted into SCID mice using 24 human hematopoietic cell lines. SCID mice were inoculated subcutaneously with 2x10^5 cells from each cell line and observed for 12 weeks. Among the six adhesion molecules examined, L-selectin increased the incidence of lymph node metastasis, and CD44 expression was related to both lymph node and organ (hematogenous) metastasis. Expression of the other adhesion molecules did not correlate with the metastatic potential of the cell lines examined. Treatment with a monoclonal antibody to the standard form of CD44 (CD44a), Hermes-3, inhibited the metastasis of CD44* cell lines. Thus, it is concluded that at least CD44a expression is important in both lymphatic and hematogenous metastasis.
Cryptococcus neoformans is a ubiquitous fungus that is a pathogen for immunocompromised patients with AIDS, malignancies, and patients who have undergone bone marrow transplants. Antifungal therapy often fails to eradicate the infection in these immunocompromised patients. We and other groups have shown that passively administered monoclonal antibody (MAb) to C. neoformans can alter the course of infection in the mouse, which may suggest that potential benefits for treating AB and antifungal therapy in humans.

We previously found that an IgG MAb with variant (15E2) was highly protective against cryptococcal infection in AB mice, whereas the parental IgG MAb had only limited protective function, but it actually decreased animal survival ("enhanced killing"). We used three immunodeficient mouse strains, the NK deficient Beige mutant and TdT cell deficient SCID mice and C57 B16 T cell knock out mice C57B16 in order to determine how antibody isotype affects immunodeficient animal survival. We found that control Beige mice were highly susceptible to cryptococcal infection compared to the normal parental C57 BLA mice. Administration of IgG anti-body prolonged animal survival significantly in both Beige and C57 BLA mice, whereas IgG MAb still decreased animal survival in both strains. This suggests that other antibody properties other than "enhanced killing" is mediated through NK cells. We also found that both SCID and C57B16 mice were more susceptible to cryptococcal infection than their parental strains. The SCID and C57B16 susceptibility was much less marked than encountered within the Beige mouse strain. As expected, the parental C57 BLA mice were protected by IgG when infected, but surprisingly, SCID and C57B16 mice were not, whereas the "enhanced killing" by IgG administration still remained. This suggests that IgG MAb protection is mediated through the T cells whereas IgG "enhanced killing" is mediated by yet another mechanism.