

ABSTRACTS

ORAL PRESENTATIONS

ABSTRACTS - Fourth International Conference on Malignant Lymphoma, Lugano

- 1** NATURE OF STERNBERG-REED CELLS AND OTHER BIOLOGICAL PROBLEMS.
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The cellular origin and clonal status of SR cells is still an enigma. It will be reported on immunophenotypical studies of frozen and paraffin sections with cell type-specific and cell type-characteristic monoclonal antibodies, as well as on chromosomal analysis and molecular genetic studies using Ig and T cell receptor gene and EBV DNA-specific probes. The results show that SR cells are clonal and phenotypically resemble late, i.e. activated lymphoid cells, and genotypically resemble early, i.e. immature lymphoid cells. In addition, there is evidence that SR cells harbor the EBV genome in more than 50% of the cases. These findings support the notion that SR cells represent the malignant element in Hodgkin's disease, that these cells are lymphocytic in origin, and that EBV is involved in the pathogenesis of a significant number of cases of Hodgkin's disease.

- 2** LOW SERUM INTERLEUKIN-2 RECEPTOR (sIL-2R) LEVELS CORRELATE WITH A GOOD PROGNOSIS IN PATIENTS WITH HODGKIN'S DISEASE (HD). A. Gause, V. Roschansky, A. Tschiersch, R. Schmits, V. Diehl, M. Pfreundschuh. Medizinische Universitätsklinik, University of Cologne, D-5000 Cologne 41, F.R. Germany

In order to evaluate the clinical significance of sIL-2R levels in the serum of patients with HD, we tested the pretreatment sera of 82 patients with HD. The HD patients had a significant elevation of sIL-2R compared to normal controls (7748 U/ml vs 273 U/ml, $p < 0.001$). In patients presenting with B-symptoms, the median receptor level was significantly higher than in patients without B-symptoms (4577 vs. 2045 U/ml; $p < 0.01$). Pts. in stage IVB had the highest sIL-2R levels (8077 U/ml) followed by stage IIIB (6740 U/ml) and IIB (3360 U/ml). Of 67 pts. evaluable for response, all patients with low levels of sIL-2R (< 1000 U/ml) achieved complete remission and no relapses occurred in this group after a median time of observation of 12 months. Patients with very high sIL-2R ($> 10\ 000$ U/ml) had a significantly lower CR rate (8/15 = 53%) compared to the group with intermediate (1000 -10 000 U/ml) sIL-2R levels (40/56 = 78%) and the group with low levels (< 1000 U/ml). Progressing disease also correlated with sIL-2R levels: while it was zero in pts. with IL-2R < 1000 U/ml, it was 15% in the intermediate and 40% in the group with high sIL-2R levels. We conclude that the determination of pretreatment serum levels of sIL-2R allows for the discrimination of prognostic subgroups which might be helpful for the design of more custom-tailored therapy programs for this disease.

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3 AN EPIDEMIOLOGIC VIEW OF THE NEW CYTOGENIC FINDINGS IN HODGKIN'S DISEASE. N.E. Mueller, Harvard School of Public Health, Boston, MA, USA

The pathogenesis of Hodgkin's disease (HD) has been a matter of much speculation for clinicians, epidemiologists, and basic scientists for many years. This interest is triggered by the unusual clinical features of the disease suggesting an admixture of a chronic inflammatory process with a malignancy. Recent advances in molecular biology provide insight into underlying genetic events. What emerges is a pattern of four recurrent gene rearrangements. These consist of the α and β chain genes of the T-cell receptor and the heavy and light chain immunoglobulin genes of B-cells. All four share a common mechanism of random gene rearrangement during lymphocyte maturation upon immune stimulation. These gene rearrangements parallel the chromosomal abnormalities seen in HD.

Such cytogenetic findings are consistent with the complex immune dysfunction seen in patients, which appears to be secondary to chronic endogenous exposure to IL-2. Taken together, the picture which emerges is that the pathogenesis of HD involves a sustained host response to chronic tissue-based antigenic stimulation.

This interpretation is supported by the epidemiologic features of HD which suggest that HD may develop as a rare consequence of infection with a common latent virus where risk is increased if infection is delayed until adolescence or young adulthood. Such "late" infections are generally more clinically severe and may result in more chronicity of virus replication. Serologic studies of antibodies against the Epstein-Barr Virus - a candidate agent - in HD patients support this hypothesis.

In summary, the new cytogenetic findings in HD converge with previous epidemiologic, immunologic, and clinical data to support a unifying hypothesis of pathogenesis, in which the gene rearrangements occur secondarily to chronic stimulation of T-cell receptor and immunoglobulin genes.

4 NON HODGKIN'S LYMPHOMA ARISING IN PATIENTS TREATED FOR HODGKIN'S DISEASE IN THE BNLI - A 20 YEAR EXPERIENCE. M. H. Bennett, K. A. MacLennan, G. Vaughan Hudson and B. Vaughan Hudson. British National Lymphoma Investigation. Dept. of Oncology. UCMMS. The Middlesex Hospital. Mortimer Street. London. W1N 8AA.

Between 1970 and 1989 over 3,000 patients have been entered into British National Lymphoma Investigation (BNLI) clinical trials of Hodgkin's disease (HD). Of these patients, 24 have subsequently developed immunophenotypically confirmed Non Hodgkin's Lymphoma (NHL). In all cases the original sections upon which the diagnosis of HD was made have been reviewed and in the majority immunocytochemistry has been performed on additional paraffin sections. In two patients the original diagnosis of HD has been changed to one of T cell rich B cell lymphoma and in both the new lymphoma was of diffuse large B cell type. For the remaining 22 cases the diagnosis of HD was confirmed upon review; 8 being classified as lymphocyte predominant (LP), 8 as nodular sclerosis (NS) and 6 as mixed cellularity (MC). Nine patients had Stage III or IV disease at presentation and eleven had B symptoms. Twelve were treated by chemotherapy, eight by radiotherapy (RT) alone and two by RT with chemotherapy for early relapse.

No correlation was observed between the original histological subtype of HD and the subsequent NHL. Of the 8 patients with LP, 5 developed high grade B cell NHL and 3 peripheral T cell NHL (all presented with the nodular subtype of LP). In the 8 patients with NS, 7 developed B cell NHL (2 low grade and 5 high grade) and 1 a peripheral T cell lymphoma. All 6 patients with MC developed B cell NHL (4 low grade and 2 high grade).

The 6 low grade B cell lymphomas (4 follicular and 2 diffuse) all developed within 3 years of the original diagnosis of HD, 2 being synchronous presentations. The 12 high grade B cell NHL developed between 5 and 18 years following the diagnosis of HD and the 4 peripheral T cell NHL at periods from 4 to 16 years following presentation with HD.

The results suggest that these patients have a propensity for lymphoproliferative disorders, possibly associated with some immune deficiency and that the subsequent development of NHL is not treatment related. The findings also emphasise how important it is to biopsy recurrent disease.

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HODGKIN'S DISEASE AND ITS RELATION TO NON-HODGKIN'S LYMPHOMA. M.-L. Hansmann. Department of Pathology, Michaelisstraße 11, D-2300 Kiel 1, FRG

The differentiation of Hodgkin's disease into 4 types according to the Rye Conference (LUKES et al. 1966) is generally recognized and applied in the diagnostics of pathologists. In recent years, however, it has become evident that some types of HD should perhaps be regarded as B-cell neoplasias and others as T-cell derived lymphomas. Arguments for the B-cell derivation of Hodgkin and L&H cells in nodular paraneoplasia are the reactivity with B-cell monoclonal antibodies such as Ki-B5 and L26 as well as the expression of J-chains. The infiltrates are mainly composed of B lymphocytes and show large networks of follicular dendritic cells. The diffuse paraneoplasia shows a comparable marker profile of L&H and Hodgkin's cells as that of the nodular type. However, in contrast to the nodular variant, the diffuse type shows a relatively high number of T lymphocytes and only small follicular dendritic cell networks. The latter could be demonstrated with monoclonal antibodies working on paraffin sections (2G7, Ki-FDC1P). During differentiation of the T-cell subsets in stage I of nodular paraneoplasia statistically relevant, high numbers of Leu7+ cells could be found. The distribution of T and B lymphocytes as well as the number of follicular dendritic cells in mixed type of Hodgkin's disease with a high content of lymphocytes is often similar to diffuse paraneoplasia. In contrast to diffuse paraneoplasia the mixed type shows typical Sternberg-Reed cells usually positive for CD15 and CD30 monoclonal antibodies, which do not react with most cases of paraneoplasia.

Because it has been shown that Hodgkin's disease of nodular and diffuse paraneoplasia subtypes has a much better clinical prognosis than that of mixed type with a high content of lymphocytes, the distinction between these subtypes has not only biological but also clinical relevance. Paraneoplasia, particularly the nodular type, sometimes undergoes a transition to large-cell lymphomas of B type (centroblastic lymphomas), having a much better prognosis than high-grade B-cell lymphomas secondary to low-grade B-cell non-Hodgkin's lymphomas. One of 14 such cases examined, however, showed a positive reaction for CD15 and CD30. In contrast to paraneoplasia, mixed type of Hodgkin's disease can develop into T-cell lymphomas mainly of large-cell anaplastic type. The immunophenotyping of Hodgkin's disease is of importance in the differentiation of biologically distinct entities showing different clinical behaviors.

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PERIPHERAL T CELL LYMPHOMAS.

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The development of T cells from stem cells to effector cells results from a two-wave process of proliferation and differentiation. The cells of the first differentiation wave are the precursor T cells, and those of the second differentiation wave are peripheral T cells. In the first differentiation wave, resting/circulating antigen-reactive T lymphocytes are produced which differ from each other in their antigen receptor-specificity. In the second differentiation wave, those T lymphocytes multiply whose antigen receptors have found the corresponding antigen. Thus three major forms of differentiation can be distinguished in the peripheral T cells: 1) resting/circulating T cells; 2) activated T cells; 3) effector T cells. In addition, there are probably at least two major organ-specific sublines of peripheral T cells, i.e. nodal T cells and mucosa-associated T cells. There also might be T cells which fulfill their function in the skin.

All the above mentioned T cell sublines and differentiations forms can be associated with certain lymphoma types, i.e. lymphomas of T cell type can be divided into categories of precursor T cell lymphomas and peripheral T cell lymphomas. The peripheral T cell lymphomas can be subdivided into lymph nodal, mucosal, and cutaneous T cell lymphomas. The gut mucosal T cells lymphomas are often associated with enteropathy. The lymph node, mucosal, and cutaneous T cell lymphomas can be further subdivided into those in which all tumor cells are similar to recirculating resting (non-activated T cells) and into those which resemble activated T cells. The latter group consists of Ki-1+ (CD30+) anaplastic large cell lymphomas, angioimmunoblastic type T cell lymphoma, pleomorphic T cell lymphoma of large cell type, and lymphomatoid papulosis. Some of the peripheral T cell lymphomas appear to contain virus-specific DNA sequences.

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- 7** CURRENT EPIDEMIOLOGIC AND THERAPEUTIC SITUATION IN AIDS.
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Several dideoxynucleosides, including 3'-azido-2',3'-dideoxythymidine, (zidovudine, azidothymidine, AZT), 2',3'-dideoxycytidine (ddC), and 2',3'-dideoxyinosine (ddI), have been shown to be potent inhibitors of human immunodeficiency virus (HIV) replication in human T cells and macrophages. These compounds undergo anabolic phosphorylation within target cells to a 3'-triphosphate moiety; as triphosphates, they act at the level of HIV DNA polymerase (reverse transcriptase). AZT has been shown to reduce the morbidity and mortality of patients with severe HIV infection and to at least temporarily ameliorate certain cases of HIV-induced dementia. AZT is one factor in the decrease of actual (versus expected) cases of fulminant AIDS in the U.S. In Phase I studies, ddC and ddI have been shown to induce immunologic and virologic improvements in some patients with AIDS or related disorders; Phase II studies of ddC and ddI are underway. The use of these drugs can be associated with serious toxicity. AZT can cause bone marrow toxicity or myositis with prolonged use; ddC can cause peripheral neuropathy; and ddI can cause sporadic pancreatitis and peripheral neuropathy. However, for each compound, a therapeutic window likely exists: an anti-HIV effect may be attained without short-term toxicity in many patients. Dose-intensity appears to be an important determinant of the toxicity of dideoxynucleosides. Studies are underway to explore how the therapeutic profiles of these compounds may be enhanced by attention to scheduling or through the use of combination therapy.

- 8** QUANTITATIVE MAGNETIC RESONANCE STUDIES OF LUMBAR VERTEBRAL MARROW IN PATIENTS WITH REFRACTORY OR RELAPSED HODGKIN'S DISEASE. S.Smith, C.Williams, R.Edwards, J.Davies. Magnetic Resonance Research Centre & Department of Haematology, Liverpool University, P.O Box 147, Liverpool, U.K. L69 3BX.

Elevated lumbar vertebral marrow proton relaxation times (T_1 and T_2) have been reported in various bone marrow disorders¹. Magnetic resonance (MR) imaging has been shown to be of value in detecting focal marrow infiltrates², however the role of quantitative MR is as yet unclear. The aim of this study is to evaluate the role of quantitative MR in patients undergoing autologous bone marrow transplantation (ABMT) for Hodgkin's disease (HD).

20 consecutive patients with refractory or relapsed HD referred for ABMT had quantitative MR studies of the lumbar spine and bilateral iliac crest trephines performed as part of their pre-transplant work up. MR studies were performed on a 1.5 Tesla G.E. Signa system using a predefined protocol¹, total imaging time was 45 minutes. 15 age and sex matched controls were also studied.

Significantly prolonged lumbar vertebral marrow T_1 times were found in 6 patients compared to controls ($p \leq 0.01$), consistent with marrow involvement with lymphoma. Only two of these patients having evidence of marrow involvement with HD on bone marrow trephine. T_1 variation was much greater in these 6 patients (% CV 36-86%) when compared with the 14 other patients with normal iliac crest trephines (%CV 9-20), or controls (%CV 7-18), reflecting the focal nature of the marrow involvement in HD.

4 of the 6 patients with abnormal pretransplant MR studies were examined 11 weeks post ABMT. All showed normalisation of mean lumbar vertebral marrow T_1 and T_1 variation with treatment, suggesting a good response to therapy.

Quantitative MR studies of lumbar vertebral marrow provide an objective and non-invasive means of documenting marrow involvement with lymphoma, and allowing the assessment of treatment response. MR studies of marrow combined with bone marrow trephine may improve patient selection for procedures such as ABMT.

1. Smith SR, Williams CE, Davies JM, Edwards RHT. Radiology 1989;172:805-810.

2. Shields AF, Porter BA, Olson DO, et al. J Clin Oncol 1987; 5: 225-230.

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MOLECULAR GENETICS OF HUMAN B CELL NEOPLASIA.

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In Burkitt lymphoma, a chromosome translocation juxtaposes the c-myc proto-oncogene to one of the loci for immunoglobulin genes. This juxtaposition results in the deregulation of transcription of the c-myc oncogene, leading to malignant transformation. It is becoming clear that in most endemic Burkitt lymphomas the t(8;14) chromosome translocation occurs during physiologic V-D-J joining while in most sporadic Burkitt lymphomas the translocation occurs during heavy chain switching. We have studied the molecular basis of high grade lymphomas in patients with AIDS and found that most EBV positive lymphomas have the same molecular rearrangements of endemic Burkitt lymphomas, while the EBV negative lymphomas have the same molecular rearrangements of sporadic Burkitt lymphomas. We have also identified, cloned and characterized bcl-2, the gene involved in most follicular lymphomas. This gene is transcribed into 3 mRNAs and codes for two protein products, alpha and beta, 26 kb and 22 kd in size respectively. The alpha product is located on the inner surface of the cell membrane and may be involved in signal transduction in B cells. In approximately 70 % of follicular lymphomas the rearrangements occur within a short segment of DNA at the 3' untranslated region of the gene. In approximately 10-20 % of cases the rearrangements occur in a region approximately 15 kb 3' to the gene. Interestingly, we recently discovered rearrangements in the 5' flanking region of the bcl-2 gene in B-CLL. These rearrangements involve recombination of the bcl-2 locus with either the immunoglobulin lambda locus or kappa locus. Approximately 10-20 % of B-CLLs carry rearrangements of the 5' flanking region of bcl-2 with either the lambda or kappa locus.

Recently we have discovered that chromosome translocations are also involved in the progression of B cell tumors and that low-grade B cell tumors with the t(14;18) chromosome translocation progress to high grade tumors following a t(8;14) chromosome translocation. We have also discovered an additional locus, bcl-3, capable of deregulating c-myc in B cell neoplasms. Probes specific for these DNA rearrangements and amplification methods can be used for the diagnosis, prognosis and monitoring of human B cell neoplasms.

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THE CONTINUING CHALLENGE OF HODGKIN'S DISEASE.

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Patients with Hodgkin's disease, treated at most major medical centers, enjoy a cure rate, on the average of approximately 75 percent. An additional five or ten percent will not die of Hodgkin's disease, because of the success of secondary treatments or they succumb to other related or unrelated causes. There are subgroups of patients who fare better or worse than this average, depending on prognostic factors such as age, stage, bulk or site of disease and on the primary management program.

Substantial improvements in these curability and survival statistics will be difficult to achieve and demonstrate. The major efforts of clinical investigators of Hodgkin's disease in 1990 are to identify and reduce the serious long term morbidities of treatment programs and to assure that the excellent outcome results achieved at major centers can be accomplished more widely throughout the world.

The emerging challenge for investigators of Hodgkin's disease in the next decade is not the refinement of treatment methods, but a better understanding of the nature, etiology and pathogenesis of the disease.

There are very important data and observations that Hodgkin's disease is not a single disease entity based on epidemiological, histologic and immunologic characteristics. Despite the heterogeneity of the disease, familial clustering and HLA correlations give strong evidence that there is a genetic basis for at least a component of the pathogenesis.

The new tools and concepts of the molecular geneticist combined with the recognitions of more homogeneous disease subgroups give great promise that the genetic basis of Hodgkin's disease will soon be understood.

Then, as with other diseases identified as genetic in origin, the challenge will be to know whether a single or multiple genes are involved, what is the role of environmental factors, what gene products (or their absence) are responsible for the disease, and finally how to prevent or reverse the disorder on a rational, rather than empiric basis.

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- 11** ALTERNATING VERSUS HYBRID MOPP-ABVD IN HODGKIN'S DISEASE. THE MILAN EXPERIENCE. Bonadonna G., Viviani S., Santoro A., Bonfante V., Zanini M., Negretti E., and Valagussa P. Istituto Nazionale Tumori, Milano 20133, Italy.

Our long-term results on stage IV Hodgkin's disease confirm that MOPP alternated with ABVD (MOPP-ABVD) is superior to MOPP (Proc. ASCO 1989, Abstr. 976). To meet more closely the requirements for the Goldie and Coldman assumption to work, in July 1982 we started a randomized trial testing MOPP-ABVD through two different sequences in stage IB-IIA bulky, IIB, III (A and B), and IV. One arm consisted of monthly alternating one cycle of MOPP and one cycle of ABVD; in the other arm half-cycle of MOPP was alternated with in 1 month period with half-cycle of ABVD (hybrid). Treatment was given to CR plus 2 consolidation cycles (minimum 6 cycles). RT (25-30 Gy) was delivered only to site(s) of initial bulky disease. Of 381 cases enrolled into the study, for present analysis 300 entered within December 1987 (i.e. with a minimum follow-up of 20 months) are evaluable. With a median follow-up of 4 years, the comparative 5-years results (in %) are as follows:

	Alternating	Hybrid
Complete remission	89	88
Freedom from progression	64	69
Relapse free survival	71	77
Total survival	79	79

Stage, age, B symptoms, and histology failed to influence the attainment of CR. In patients with stages II and III relapsing after CR, 33 of 42 (79%) recurred only in nodal sites and 9 of 42 (21%) in extranodal + nodal sites. Present findings suggest: (1) MOPP-ABVD delivered through different sequences yielded comparable treatment outcome; (2) in stage IV our prior results with MOPP-ABVD are confirmed; (3) in stages II and III both role and extent of RT combined with chemotherapy should be reconsidered.

- 12** LONG TERM TOXICITY OF HODGKIN DISEASE TREATMENT
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Based on the experience of the EORTC trials (1963-1988), the following late effects of HD therapy were studied:

- *Late injuries of the gastro-intestinal tract* (ulcer, gastritis, and small bowel injury) were observed in 41 patients in first complete remission out of a series of 775 patients entered in the H2 and H5 EORTC trials (5.3%). These injuries were significantly favoured by a previous staging laparotomy ($p < 0.001$) and paraaortic irradiation ($p < 0.001$). When these two factors were considered in conjunction with the radiation fraction size used, the following results were observed:

previous laparotomy	paraaortic irradiation	fraction size (Gy)	patients at risk	injuries #	5-yr cumulative incidence rate
no	no	-	133	1	< 1%
yes	no	-	123	1	1%
no	yes	2.0	183	1	< 1%
no	yes	2.5	48	3	8%
no	yes	3.3	34	3	11%
yes	yes	2.0	176	18	8%
yes	yes	2.5	48	5	12%
yes	yes	3.3	30	9	28%

- *Late functional pulmonary impairment* was prospectively investigated in the H6-U trial (1981-1988) which compared 3 MOPP-mantle field irradiation-3 MOPP to 3 ABVD-mantle field irradiation-3 ABVD for unfavourable early stages HD. At the end of treatment, vital capacity (VC) rate as compared to the theoretical value was found to be significantly lower in the ABVD arm (76% vs 86%, $p < 0.005$). At 1 and 2 years, a slight difference in VC was still observed, favouring the MOPP arm, but was no more statistically significant. Data also suggested that high irradiation dose and fraction size greater than 2.0 Gy could play an additional role in late pulmonary toxicity.

- *Myocardial infarctions (MI)* were observed in 21 patients (17 males and 4 females) out of 1,048 patients treated on the H1, H2, and H5 EORTC protocols (2%). Overall MI occurred 3 to 225 months posttreatment completion giving a 10-year cumulative incidence rate of 2.4%, and a 15-year rate of 4.6%. Of these 21 patients, 16 died from MI, all being males. The standardized mortality ratio (SMR) was equal to 3.8 ($p < 0.001$). Depending on age at diagnosis of HD, SMR was 14.9 ($p < 0.001$) in patients < 40 years, while in older patients it was 6.6 ($p < 0.001$). In this series, apart from age at HD diagnosis ($p < 0.01$) and sex ($p < 0.05$), no factor were found prognostic, all patients being previously irradiated in the mantle field. Nevertheless, data from Institut Gustave Roussy strongly suggest that the risk for developing a MI is closely related to mediastinal irradiation.

- *Second cancers (SC)* were diagnosed in 63 patients out of 1,579 entered in the 4 successive EORTC trials. There were 17 acute non-lymphocytic leukemias (ANLL), 13 non-Hodgkin lymphomas (NHL), and 33 solid tumours (ST). In this series, the 15-year cumulative incidence rates of ANLL, NHL, and ST were 2.3%, 1.9%, and 8.8%, respectively. The risks of SC relative to the general population incidence rates were significantly increased for both ANLL (RR=37, $p < 0.001$), NHL (RR=33, $p < 0.001$), and ST (RR=1.7, $p < 0.005$). Several factors were identified to be associated with an increase in risk of SC: a combination of radiation and MOPP for ANLL and NHL; age > 40 years for NHL and ST; and extended field irradiation for ST.

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13 A RANDOMISED STUDY OF ADJUVANT MVPP CHEMOTHERAPY AFTER MANTLE RADIOTHERAPY IN PS IA-IIB HODGKIN'S DISEASE. 10 YEAR FOLLOW UP. H Anderson, D Crowther, D Deakin, WDJ Ryder, Christie, Hospital, Manchester

From Oct.1974-Aug.1981, 115 patients with untreated supradiaphragmatic pathologically staged IA-IIB Hodgkin's disease (HD) were entered into a randomised study of adjuvant MVPP (mustine, vinblastine, prednisolone, procarbazine) or follow up alone after mantle radiotherapy.

The median duration of follow up is 131 months. 56 patients were treated with radiotherapy (RT) alone and 59 received RT plus 6 cycles adjuvant MVPP. Patient characteristics between the two groups (age, sex, stage, Karnofsky performance, bulk disease, histology and number of sites involved) were not significantly different. 113 patients achieved a complete remission (CR) with RT alone. The 10 yr survival corrected for intercurrent death was 92%. There were 9 (8%) deaths from HD (5 received RT alone). Eight patients (7%) developed a second malignancy of whom 6 have died. There have been 4 other intercurrent deaths.

The 10 year relapse-free survival (RFS) was 80% overall, 90% in the adjuvant MVPP group and 66% in the RT group ($p=0.0004$). There were 25 relapses, 19 received RT alone and 6 received adjuvant MVPP. Of the relapsed patients one progressed and died on adjuvant MVPP, one relapsed and died of infection before therapy could be given. The other 23 received chemotherapy ± radiotherapy, 19 (83%) obtained a CR and 4 progressed and died of disease. Of the CR patients, 3 died in second relapse, 3 had intercurrent deaths and 13 (68%) continue in CR.

Univariate analysis showed the following factors influenced survival - pruritus $p=0.0014$, night sweats $p=0.0016$, B symptoms $p=0.0023$, bulk $p=0.0041$, monocyte count $>0.5 \times 10^9/L$ $p=0.0059$, stage IIB $p=0.0157$, histology Mixed cellularity $p=0.0227$, lymphocyte count $<1.7 \times 10^9/L$ $p=0.0385$. Univariate analysis showed that treatment - RT only $p=0.0004$, lymphocyte count $<1.7 \times 10^9/L$ $p=0.0011$ and B symptoms $p=0.0269$ predicted relapse. Multivariate analysis was only possible for relapse as there were too few events to analyse for HD deaths. Analysis of 24 variables showed only 3 variables predicted relapse, treatment-RT alone $p=0.0001$, lymphocyte count $<1.7 \times 10^9/L$ $p=0.0005$, and haemoglobin $<14G/L$ $p=0.0191$.

The current status in the RT only group is that 45 (80%) patients are alive in CR, 5 (9%) have died of HD and 6 (10%) had intercurrent deaths. In the adjuvant MVPP group 51 (86%) are alive in CR, 4 (7%) died of HD and 4 (7%) died of intercurrent deaths. Adjuvant MVPP has shown a significant improvement in RFS but not overall survival.

14 EVALUATION OF THE TOXICITY OF THREE COURSES OF ABVD AND MEDIASTINAL IRRADIATION IN FAVORABLE HODGKIN'S DISEASE.

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The association of chemotherapy and radiotherapy in Hodgkin's disease has been associated with iatrogenic effects. 40 patients (pts) have been studied to evaluate the early toxicity following 3 courses of ABVD (cumulated dose of Adriamycin 150 mg/m^2 and Bleomycin 60 mg) and mediastinal irradiation. Mantle field irradiation was performed one month after the last chemotherapy course at the dose of 40 grays. The fraction size was 2, 1 and 2, 2 grays; anterior and posterior fields were used with a 18 MV photon accelerator.

Cardiopulmonary toxicity was assessed by dimensionnal echocardiogram, thoracic scan and pulmonary functionnal tests, they were performed from 6 months to 3 years after completion of irradiation.

The 40 pts (21 women, 19 men), (mean age : 33 years), were in complete remission and 6 experienced dyspnea on exertion.

Initial staging showed : stage I : 5 pts ; stage II : 29 pts ; stage III_A : 6 pts.

Cardiac toxicity : the left ventricular ejection fraction ranged from 50 to 77 % (mean 63 %), 8 pts had a minor pericardial effusion ; 4 pts had valvular calcification ; 8 pts had minimal cardiac abnormalities.

Pulmonary toxicity : CT scans showed small pleural effusion with pleural thickening in 19 pts (only one was symptomatic) ; mediastinal fibrosis in 7 pts and apical fibrosis in 8 pts. The total pulmonary capacity value was abnormal ($< 80\%$) in 19 pts (50%) and change in CO diffusion ($< 70\%$) was found in 10 cases. In this serie, we can conclude that early cardiac toxicity was absent despite the use of Adriamycin and mediastinal irradiation. Pulmonary toxicity was more frequent and may be due to the fraction size used which was larger than 2 grays.

In conclusion, 3 courses of ABVD chemotherapy appears safe, pulmonary lesions may decrease with shorter radiotherapy fraction size.

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- 15** COMBINATION CHEMOTHERAPY WITH CHLORAMBUCIL, VINBLASTINE, PREDNISOLONE AND PROCARBAZINE (ChLVPP) IN HODGKIN'S DISEASE: 14 YEAR FOLLOW UP ON 284 PATIENTS. T J McElwain, P Patel, P Selby, Cancer Research Campaign, Section of Medicine, Institute of Cancer Research and Royal Marsden Hospital, Sutton, Surrey SM2 5PT, UK.

Two hundred and eighty four patients with advanced Hodgkin's disease (HD) (Stage II with poor prognostic features and stage III/IV) have been treated with the ChLVPP combination chemotherapy regimen (chlorambucil, vinblastine, procarbazine and prednisolone) in a single-centre unselected series. Median follow up is 92 months. Fifty five patients had previously received radiotherapy. Eighty five percent of previously untreated patients and 91% of previously irradiated patients entered complete remission (CR); 71% and 68% of these respectively remained in CR at 10 years and 65% and 64% of each group respectively are alive at 10 years. On univariate analysis, age, stage, site of visceral disease and lymphocyte count predicted survival and on multivariate analysis, age, absence of symptoms, absence of lung, liver or bone marrow disease and achieving a CR remained important predictors of survival. Acute toxicity was mild. The 10 year actuarial risk of acute leukaemia was 2.7%.

This study adds support to the view that chlorambucil is as effective and less toxic than mustine in combination chemotherapy for HD and that MOPP chemotherapy need no longer be used.

- 16** MOPP vs ABVD vs MOPP alternating with ABVD as treatment for advanced Hodgkin's disease: Results at a median follow-up of 4 years. JR Anderson, GP Canellos, KJ Propert, R Cooper, N Nissen, KH Antman, and AJ Gottlieb for the Cancer and Leukemia Group B, Brookline MA, USA.

369 eligible patients (pts) with previously untreated Hodgkin's disease, Stages III₂A-IVB or in relapse after primary radiotherapy (RT) for early stage disease, were randomized to receive 28 day cycles of MOPP (HN₂ 6 mg/m² and vincristine 1.4 mg/m² {max, 2 mg} days 1 and 8, procarbazine (PCZ) 100 mg/m²/d x 14; prednisone 40 mg/m²/day x 14], ABVD [adriamycin (ADR) 25 mg/m², bleomycin 10 mg/m², vinblastine (VBL) 6 mg/m² and DTIC 375 mg/m² days 1 and 15] or MOPP alternating with ABVD (M/A) after stratification for age, stage and prior RT. Pts treated with MOPP or ABVD were treated until complete response (CR) plus 2 cycles (min 6), whereas M/A pts received 12 cycles. Pts with stable or progressive disease after 2 cycles or not reaching CR after 6 cycles of MOPP or ABVD were to be crossed-over to the alternative regimen. Median age was 34, 63% were male, 45% were Stage IV and 17% received prior RT. Complete response rates to initial therapy were: MOPP, 66%; ABVD, 83%; M/A, 76% (p=0.04). Estimated failure-free survival (FFS: defined as time to 1st progression, relapse or death from date of entry) at 5 years was: MOPP, 47%; ABVD, 57%; M/A, 66% (p=0.004). Survival at 5 years was estimated to be: MOPP, 66%; ABVD, 74%; M/A, 76% (p=0.21). A dose analysis of 49 pts on each regimen showed that by cycle 3 of MOPP 44%/39% of pts received full protocol dose of HN₂/PCZ (due to toxicity) whereas 80%/74% of ABVD pts received full dose ADR/VBL. 74%/70% of M/A pts received full dose HN₂/PCZ in cycle 3 (MOPP). Cross-over data available from 84 pts [50 MOPP=>ABVD, 34 ABVD=>MOPP] showed a 2nd CR rate of 36% after MOPP failure vs 62% after ABVD failure (p=0.02). However, no difference in FFS from cross-over exists between MOPP and ABVD cross-overs: only 24% of pts in both cross-over groups remain free of second failure at 3 years from cross-over. Overall ABVD and M/A provide a FFS advantage over MOPP: further follow-up is required to assess potential differences in survival.

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17 MANAGEMENT OF RELAPSE AND SURVIVAL IN ADVANCE STAGES HODGKIN'S DISEASE (HD): THE EORTC EXPERIENCE. J.M.V. Burgers, R. Somers, M. Henry-Amar, M. Tarayre, P. Carde, J. Thomas, A. Hagenbeek, M. Monconduit, B.E. De Pauw, W.P.M. Breed, L. Verdonck, M. Hayat, R. Zittoun. Antoni van Leeuwenhoek Huis, 1066 CX Amsterdam, The Netherlands.

From 1981 to 1986, 207 patients with clinical stage IIIB-IV HD were treated by 2 courses of MOPP, and then randomised to receive MOPPx6 (M arm) or alternating ABVDx2/MOPPx2/ABVDx2 (M/A arm). Thereafter, iceberg radiotherapy (IRX) was given to original nodal bulks (> 5cm) or to sites not in complete remission (CR) after 4 courses. The dose delivered was 20 Gy if CR at time of IRX, or 30 Gy in other cases. Remission status was evaluated after 2, 4, and 8 courses, and after IRX. Data were up-dated December 1, 1989.

Overall, 4 (2%) patients had progressive disease before randomisation, 6 (3%) patients refused randomisation and 5 (2%) patients were not randomised for other reasons. Of the 192 (93%) remaining patients, 96 were included in the M arm and 96 in the M/A arm. At the end of treatment, the CR rates were 69% and 73% in the two arms respectively. The 6-year FFP rates were 45% and 60% (p=0.02), and the 6-year survival rates were 57% and 68% (p=0.09), respectively.

A progression while under treatment occurred in 42/207 (20%) patients; 21 (10%) patients relapsed within 18 months post-treatment start, and 25 (12%) patients at a greater interval (maximum 65 mo). Localisation of progression or relapse and therapy given were detailed in 63/88 (72%) patients (ie 27 progressions and 36 relapses). Localisations were: nodal areas only, 33 patients (3 after previous IRX); nodal areas with lung or bone extension, 11 patients; relapse in isolated organs, 7 patients; multiple nodal and extranodal sites, 12 patients (included 8 progressions). Among the 27 patients with progression, RX alone or in combination with chemotherapy was given in 7 of whom 6 patients reached a CR and 4 patients survived. Among the 36 patients who relapsed, 23 achieved a 2nd CR of whom 15 patients were given RX and 3 patients ABMT.

Data available	2nd CR	Relapse	Alive (4-year rate)
Progression n=27	6 (22%)	1/6	4 (8%)
Relapse n=36	23 (64%)	11/23	20 (53%)
All patients n=88			32 (34%)

Conclusion: At longer follow-up the survival advantage for the M/A arm seems less convincing. Progressions and relapses occur mostly in non irradiated nodal areas. Radiotherapy as part initial management could probably contribute to improve survival in these patients.

18 SALVAGE RADIOTHERAPY IN RECURRENT HODGKIN'S DISEASE. M. Brada, R. Eeles, J. Nicholls, S. Ashley, M. J. Peckham, A. Horwich. Academic Unit of Radiotherapy & Oncology, The Institute of Cancer Research and The Royal Marsden Hospital, Downs Road, Sutton, Surrey, SM2 5PT. England

Forty-four patients with Hodgkin's disease which relapsed after chemotherapy were treated with salvage radiotherapy (S-RT) with curative intent (1973 - 1989). Patients were aged 7 to 80 years (median 32 years) at the time of S-RT and the median follow-up from S-RT was 4 years (1 - 15). The staging at the time of initial presentation was: IA-2, IB-1, IIA-3 (infradiaphragmatic-2 and IIAE-1), IIB-4, IIIA-8, IIIB-13, IVA-5, IVB-8. Patients either recurred following first-line or subsequent chemotherapy or had disease refractory to it. Salvage therapy consisted of radiotherapy alone in 16 and combined chemotherapy and radiotherapy in 28 patients. The overall CR rate of salvage therapy was 72%.

The overall median survival of 44 patients was 4.5 years from S-RT with 47% 5 year and 37% 10 year survivals. Age (>40yrs) and short disease-free interval (DFI<1yr) were adverse independent prognostic factors for survival. There was no significant survival difference between patients treated by radiotherapy or combined modality therapy even when stratified by age and DFI. Of 22 deaths, 19 were due to Hodgkin's disease, and one each due to leukaemia, gastric carcinoma and myocardial infarction. There were no other treatment related deaths.

We conclude that radiotherapy with or without chemotherapy has a role in the salvage of patients failing first-line chemotherapy.

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- 19** AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR REFRACTORY OR RELAPSED HODGKIN'S DISEASE. THE MEMORIAL SLOAN-KETTERING CANCER CENTER EXPERIENCE USING HIGH DOSE CHEMOTHERAPY WITH AND WITHOUT HYPERFRACTIONATED ACCELERATED TOTAL LYMPHOID IRRADIATION. Joachim Yahalom, M.D., Subhash Gulati M.D., Ph.D. and Zvi Fuks, M.D. Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Fifty patients with advanced stage Hodgkin's disease who relapsed or failed to respond to multiple regimens of combination chemotherapy were entered onto two autologous bone marrow transplantation protocols. Twenty-eight patients who did not have prior radiation therapy were treated with protocol A. This protocol consisted of reinduction with conventional doses of combination chemotherapy followed by boost local field irradiation to areas of residual disease (1500 cGy within 5 days) and total lymphoid irradiation (2004 cGy given in 12 fractions of 167 cGy each t.i.d. delivered within 4 days). Chemotherapy consisted of Etoposide (250 mg/m²/day i.v. x3 days) and Cyclophosphamide 60mg/kg/day i.v. x2 days) followed 48 later by infusion of cryopreserved, unpurged autologous bone marrow. Twenty-two patients who have had prior radiation therapy were treated with protocol B. This protocol consisted of reinduction with conventional dose of chemotherapy followed by involved field radiation therapy (when tolerance to residual disease has not been previously reached). High dose chemotherapy regimen consisted of BCNU (250 mg/m² i.v. day 1), VP 16 (250 mg/m² i.v. days 2-4), Cyclophosphamide (50 mg/kg i.v. days 5-7) followed by autologous bone marrow transplantation.

Of 28 patients in protocol A, 5 died during the immediate peritransplant period, 22 entered a complete remission, and one had a partial response and died. Five of the 22 patients relapsed (4 within 6 months, one after 39 months) and one died. Seventeen patients (61%) are alive with no evidence of disease 7-47+ months (median-24+ month) following completion of therapy. Of the 22 patients in protocol B, 1 died of CMV pneumonitis, and 11 relapsed (6 died of disease progression). Ten patients (45%) are alive and disease-free 12-38+ months (median-22+ months) after therapy. Treatment with protocol A resulted in a high rate of complete remission, relative low relapse rate but was associated with considerable toxicity. Protocol B was less toxic but carried a higher risk of relapse. Both protocols offer a potential cure for patients with refractory or relapsed Hodgkin's disease who have exhausted conventional modes of therapy. Factors affecting response and toxicity will be discussed.

- 20** 100 CASES OF RELAPSED HODGKINS DISEASE TREATED WITH BEAM CHEMOTHERAPY AND ABMT IN A SINGLE CENTRE. A. McMillan, A.Goldstone, D. Linch, J.Gribben, R.Chopra and G. Taghipour. Dept. of Haematology, University College and Middlesex School of Medicine, 98 Chancery Mews, London WC1, UK.

We have now treated one hundred patients with Hodgkins disease using BEAM chemotherapy and Autologous Bone Marrow transplantation (ABMT). All patients had either failed induction, relapsed within 6 months of completing first line therapy or had failed 2 or more modalities of therapy. The chemotherapy doses in BEAM are BCNU (300 mg/m² day 1), cytosine (100/ 200 mg/m² bd day 2-5), etoposide 100/ 200 mg/m² od day 2-5 and high dose melphalan (140 mg/m² day 6). 54 patients had received previous radiotherapy. The first 18 patients received the lower dose of cytosine and etoposide and all subsequent patients received the higher dose. Radiotherapy to sites of residual CT abnormalities at 3 months was allowable if these sites had not been irradiated to full dose. This was carried out in 27 patients. There were 60 males and 40 females of median age 29 (range 17-54) years. At the time of transplantation the patients were classified as having disease which was primary refractory (n=15), resistant relapse (n=26), responding relapse (n=16) or untreated relapse (n=43). No patients were in remission at the time of transplantation. All patients had a bone marrow that was clear of Hodgkins disease at the time of harvesting. There were 10 procedure related deaths; of the remaining 90 patients there were 23 complete responses (CR), 54 partial responses (PR) and 13 non responses when the patients were reassessed at 3 months. The response was classed as partial if any residual CT abnormality was seen even if the abnormality did not suggest active disease. In the 66 patients with follow up for more than 15 months, 13 were in CR at 3 months and by 15 months a further 7 had attained a CR and 13 had non-progressive disease. If this latter group is included retrospectively with the CR's then the overall CR rate is 50 %. The projected overall survival at 3 1/2 years is 54 % with a relapse rate of 54 % and a median follow up of 18 months. The projected unmaintained relapse/progression free survival is 37 % at 3 1/2 years. Multivariate analysis shows that the patient sex is significant in predicting for overall survival (male 68% vs female 33%, p≤ 0.03) but the response to prior chemotherapy is not.

This study suggests that approximately 40 % of patients with refractory or multiply relapsed Hodgkins disease will be alive and free from disease progression 3 years from ABMT. Residual CT abnormalities and slow resolution of CT masses are frequent and therefore the response rate cannot be assessed for at least one year from the procedure. Longer follow up is needed to determine the proportion of the patients who will ultimately remain free from recurrent disease.

- 21** TREATMENT OUTCOME IN HODKIN'S DISEASE (HD) IN PATIENTS ABOVE THE AGE OF 60: A POPULATION-BASED STUDY. G. Enblad, B. Glimelius, C. Sundström. Departments of Oncology and Pathology, University of Uppsala, Akademiska sjukhuset, S-75185 Uppsala, Sweden.

The reasons for the poor prognosis of elderly patients with HD have not been thoroughly elucidated. Most studies concerning elderly patients are highly selected, why it can be suspected that only the "fittest ones" are included, thus excluding general conclusions. The present study included all patients with HD in three Swedish counties diagnosed between 1979-1988. During the time period, there was no special protocol designed for the elderly; in general, they were staged and treated as young patients. The aim was to analyze, in an unselected material, whether a supposed worse prognosis for the elderly could be due to differences in e.g. staging procedures, treatment intensity, poor treatment tolerance or to a more aggressive disease.

After histopathological revision, 159 of 191 cases (autopsy cases excluded) were accepted as HD, 57 (of 82) were above the age of 60 (36%, upper age 90, mean 73, median 72 years). Although staging procedures had been more intense in the young, the elderly patients tended to have more advanced stage at diagnosis, and slightly more often B-symptoms (47% vs 38%). The staging intensity did not appear to influence survival. The 5-year survival for the elderly patients was 29% compared to 85% for the young (patients dead of intercurrent disease in complete remission (CR) were not at risk after death). Radiotherapy was the primary treatment in 13 (23%) of the old and in 42 (41%) of the young patients with 5-year survival of 62% and 88%, respectively. Eight elderly patients were only palliatively treated. Thirty-four (60%) of the elderly and 56 (55%) of the young patients were treated with primary combination chemotherapy with curative intent (MOPP, MOPP/ABVD or occasionally CHLVPP/OPEC) with 24% and 84% alive at 5 years. The initial chemotherapy dose was usually "full dose". Only 7 of the elderly received >80% of the planned dose; all those patients achieved CR. Ten additional patients received CR. The majority (59%) of the elderly patients received less than 40% of planned dose intensity. The reason for dose reductions was poor patient tolerance, which often occurred early. Six patients died in treatment related toxicity, and 4 patients were severely deteriorated. Only in 5 patients could therapy, in retrospect, possibly have been given with higher dose intensity. Progressive disease during therapy was seen in 2 patients. In conclusion, tolerance to "standard" chemotherapy is very poor in elderly patients, and could be a major reason for the poor treatment outcome in these ages.

- 22** HIV-RELATED HODGKIN'S DISEASE IN 50 INTRAVENOUS USERS (IVDU). S. Monfardini, E. Vaccher, U. Tirelli et al. for the Italian Cooperative Group on AIDS-Related Tumours (GICAT). Centro di Riferimento Oncologico, 33081 Aviano, Italy

Although the natural history of AIDS seems not differ according to the risk groups, IVDU is a peculiar group of persons who often receive inadequate and delayed primary health care. HIV-related Hodgkin's disease in USA has been mainly reported in homosexual men and rarely in IVDU, while most of the clinico-pathological data collected by the GICAT from January 1984 to December 1988 concern IVDU (50 cases), in accordance to the overall epidemiology of AIDS. The only 4 homosexual men of this series will not be considered in this report. Forty-six IVDU were males and 4 were females, median age was 26 years. At presentation, persistent generalized lymphadenopathy (PGL) was diagnosed in 54% of patients (pts), AIDS in 9% and asymptomatic disease in 28%. The median number of T4 lymphocytes at diagnosis was only 264/mm³. Opportunistic infections (CI and C2) at onset were diagnosed in 20% of pts. Histology was in most pts mixed cellularity and lymphocytic depletion (76%). In almost half of cases the initial symptom was an increase of previously palpable lymph nodes due to PGL. Complete pathological staging including laparotomy or laparoscopy was performed in only 25% of pts. One case had Waldeyer's ring involvement, but no other unusual presentations were observed. After MOPP alternated or followed by ABVD or MOPP alone, CR was observed in 15/29 evaluable pts (52%) and PR in 14/29 (40%) pts. The median duration of CR was 14 months. Overall median survival was 16 months, while median survival of CRs was not reached. The median survival of pts treated with chemotherapy with initial T4 > 400/mm³ was significantly superior to that of those with T4 < 400/mm³. Pts receiving chemotherapy + radiotherapy developed opportunistic infections (28%) as well as non-opportunistic infections (21%). Death from hepatic toxicity after chemotherapy occurred in 2 pts. In conclusion, Hodgkin's disease in IVDU was not found to be associated with unusual presentations as previously reported for homosexual men, but seems to have the same less favourable outcome (lower CR rate, shorter duration of CR, shorter survival) in comparison with pts without HIV infection. Moreover less pts presented with AIDS and AIDS-related complex (ARC) in comparison to American homosexual men series. Besides opportunistic infections occurring in both risk groups, non-opportunistic infections may limit treatment administration in IVDU. Finally liver functional impairment due to chronic hepatitis often observed in IVDU may lead to unexpected and irreversible liver toxicity.

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- 23** SOME ANIMAL MODELS SUGGESTING NEW LINKS BETWEEN IMMUNOSUPPRESSION AND LYMPHOMA DEVELOPMENT. P. Ebbesen, The Danish Cancer Society, Department of Virus and Cancer, The Research Park, Gustav Wiedes Vej 10, dk-8000 Aarhus C, Denmark.

The simplicity of the animal experimental situation compared with the complexity and variability of human life makes animal experiments one way of detecting connections between immunosuppression and lymphoma development not easily observed by epidemiologic and clinical studies. Examples include lymphoma development after prolonged treatment with estrogen. This type of lymphoma development is completely preventable by syngrafting with bone marrow and other lymphoid cells from young donors. Chronic social stress in mice not caused by continued physical violence occurs in mice of low rank and also in members of animal groups where no ranking order can be upheld. In both cases the animals show enhanced susceptibility to virus leukemia development. Severe underfeeding which does not change the social order also enhances susceptibility to virus leukemia in mice. The immune status of mice in these situations is not well studied, but preliminary evidence indicates a state of immunosuppression. Another example of animal models suggesting new links between immunosuppression and lymphoma development is irradiation with ultraviolet light which is a known immunosuppressor for mice when applied to non-furred parts of the animal body. In BALB/C mice such exposure to ultraviolet light results in skin inflammation followed by leukemia development. The importance, if any, of the above animal observations for the human situation is presently undecided.

- 24** THE IMPACT OF MOLECULAR BIOLOGY ON THE DIAGNOSIS, PREDICTION OF PROGNOSIS, AND CLINICAL MANAGEMENT OF THE LYMPHOMA PATIENT. C.L. Willman, Department of Pathology and the UNM Center for Molecular and Cellular Diagnostics, UNM Cancer Center, Albuquerque, New Mexico 87131.

Scientific investigations have provided new insights into the molecular and cellular basis of many hematopoietic neoplasms. The development of molecular probes from these studies and the development of new molecular-based technologies such as *in-situ* hybridization and the polymerase chain reaction (PCR) have provided powerful new tools to study human malignancies. The utilization of molecular-based technologies in the study of lymphoproliferative lesions is already having a significant impact on:

1. Disease Diagnosis - Molecular technologies may be used to detect clonal immunoglobulin (Ig) or T cell receptor (TCR) gene rearrangements and chromosomal aberrations such as the t(14;18) or t(11;14) ("molecular cytogenetics") which may be unique to specific tumor types. Detection of the integration and/or expression of various infectious organisms (EBV, HTLV-I and II, HIV) in malignant lymphoid cells is providing new clues into tumor etiology and the potential association of some lymphomas with infectious agents.

2. Prediction of Prognosis and Biological Behavior of the Neoplasm - Morphologically indistinguishable tumors may soon be classified into distinct categories based on differing genetic abnormalities, including the analysis of the presence or absence of gene-specific mutations or deletions (in oncogenes and tumor suppressor genes) which may be associated with differing prognoses. Analysis of gene expression in lymphoid neoplasms is also providing insight into the clinical behavior and paraneoplastic phenomena associated with lymphoid tumors (such as expression of IL-5 and TGF-beta mRNA in the Reed-Sternberg cells in Hodgkin's disease).

3. Detection of Minimal Residual Disease - PCR is a remarkably sensitive tool which may be used to detect minor structural changes and rearrangements in single-copy genes in lymphoid cells. These genetic changes provide tumor markers which may be unique for each individual tumor (such as the analysis of junctional diversity at the TCR delta or gamma chain loci which may be rearranged in nearly 80% of lymphoid leukemias and lymphomas of both the T and B cell lineage) or markers which are specific for potentially neoplastic cells (such as chromosomal translocations or point mutations).

Correlative clinical and biological studies are now essential to further assess the prognostic and therapeutic significance of the highly sensitive detection of the presence of genetic abnormalities in the tumor cells of patients with malignant lymphoma.

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25 PHENOTYPIC AND FUNCTIONAL MARKERS RELEVANT TO THE DIAGNOSIS AND PROGNOSIS OF NON HODGKIN'S LYMPHOMAS (NHL). D. Delia, Istituto Nazionale Tumori, 20133 Milano, Italy.

Though the value of immunophenotype for the differential diagnosis and classification of NHL's is well established, its prognostic significance is still controversial. In general, with the better characterization of the immunological markers used for the phenotypic analysis of lymphomas (eg: cell specificity and tissue reactivity) and functional definition of the molecules identified by these markers (eg: growth factor receptors, adhesion structures), the acquisition of new parameters of biological and clinical relevance is rapidly progressing. With regard to diagnostically useful monoclonal antibodies (MoAbs), it is worthwhile mentioning those specific for lymphoid cell subsets such as CD10, positive on a large number of follicular cases and on sporadic Burkitt's Lymphoma (BL); CD5, positive on B-cell derived CLL, lymphoplasmacytoid and Centrocytic cases; CD1c (unreactive with most Centroblastic, Immunoblastic and BL cases); MoAbs specific for CD3, α and β chains of the T-cell receptor complex; for CD30 (Ki-1 and Ber-H2, the latter working also on paraffin sections) for the identification of Ki-1+ anaplastic large cell lymphomas. The number of MoAbs reactive on formalin-fixed wax-embedded sections and their use, is rapidly increasing; MoAbs specific for CD45RO (UCH-L1 and A6) and for CD45RA (4KB5 and F8.11.13) are currently used to diagnose T and B-cell lymphomas, respectively; the MoAb KP-1 for myelomonocytic lymphomas. Since adhesion molecules and lymphocyte homing receptors (LHR) may play an important role in immunosurveillance and tumor dissemination, the correlation between the expression of these molecules histological grading and clinical staging have been recently investigated. It appears that CD11/CD18 heterodimer is less expressed on high-grade NHL whereas the CD44 LHR is, among diffuse large-cell B-NHL, highly expressed on stage III/IV cases. The clinical significance of these and other LHR molecules (such as those identified by Leu8) is currently under investigation. Concerning immunological markers of prognostic value, several studies have confirmed the relevance of Ki-67 index; in addition, a recently reported marker, the MoAb Trump, reactive with an epitope of the transferrin receptor and expressed on intermediate and high grade but not low grade lymphomas, could provide an additional prognostic value.

26 HIGH FREQUENCY OF EPSTEIN-BARR VIRUS GENOME IN LYMPH NODES INVOLVED BY HODGKIN'S DISEASE IN HIV+ PATIENTS. S. Uccini, F. Monardo, A.M. Agliano, A. Gradilone, and L.P. Ruco. Dipartimento di Biopatologia Umana e di Medicina Sperimentale, Università "La Sapienza", Roma, Italy.

Persistent generalized lymphadenopathy (PGL) of HIV+ patients is characterized by hyperplastic lymph nodes with prominent activation and proliferation of B dependent areas. Some patients affected by PGL develop B non Hodgkin's lymphomas and, to a lesser extent, Hodgkin's disease, thus suggesting that PGL may be a predisposing condition. There is evidence showing that HIV is involved in the development of PGL, however HIV genome was not detected in neoplastic lymphoid cells. Epstein-Barr virus has been related to AIDS-associated B non Hodgkin's lymphomas and has been found in Reed-Sternberg and Hodgkin's cells of about 20% of cases of Hodgkin's disease in HIV negative patients. In the present study we have investigated the presence of EBV genome in 50 lymph nodes affected by PGL and in lymph nodes involved by Hodgkin's disease (HD) obtained from 7 HIV+ (HD+/HIV+) and 20 HIV negative (HD+/HIV-) patients. The presence of EBV genome was studied by in situ hybridization and Southern blot analysis. In none of the PGL lymph nodes EBV genome was found. The 7 lymph nodes involved by HD in HIV+ population were histologically diagnosed as mixed cellularity (5) and nodular sclerosis (2) type. The ratio CD4/CD8 was less than 1 in contrast to that observed in HD+/HIV- lymph nodes, in which CD4+ cells were more numerous than CD8+ cells. By in situ hybridization and Southern blot analysis, EBV genome was detected in 5 out of 7 HD+/HIV+ lymph nodes (70%); in HD+/HIV- lymph nodes EBV genome was present in 3 out of 20 cases (15%). By in situ hybridization, positivity was demonstrated in the nucleus of large cells morphologically resembling Hodgkin's and Reed-Sternberg cells. Interestingly, R-S cells were negative for C3d, which is considered the cellular receptor for EBV. In conclusion the absence of EBV genome in PGL and the high frequency in HD suggest that EBV might be involved in the neoplastic transformation. Alternatively, the high frequency of EBV genome in the HD+/HIV+ group is not associated with lymphomagenesis but might be the result of a more frequent EBV superinfection in consequence of the marked immunosuppression present in these patients due to both HIV and Hodgkin's disease.

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27 EXPRESSION OF THE BCL-2 ONCOGENE PRODUCT IN FOLLICULAR LYMPHOMA. P. Gaulard, M-F. D'Agay, N. Brousse, J. Diebold, D. Y. Mason and the GELF and GELA executive committees.

Departments of Pathological Anatomy, Henri Mondor University Hospital, Creteil; St Louis Hospital, Paris; Hotel Dieu Hospital, Paris; and the Haematology Department, John Radcliffe Hospital, Oxford.

The 14;18 chromosomal translocation, which is found in about two thirds of follicular lymphomas, brings part of the *bcl-2* proto-oncogene on chromosome 18 into juxtaposition with a portion of the immunoglobulin heavy chain gene. The breakpoint on chromosome 18 lies in the non-translated portion of the *bcl-2* proto-oncogene with the consequence that the portion coding for the protein product remains intact. We have used monoclonal antibodies raised against a peptide sequence from the *bcl-2* protein to study its expression in routinely fixed tissue sections from a series of follicular lymphomas. All cases had been entered in a multi-centre lymphoma treatment trial (either the GELF or GELA protocol) so that clinical information on patient response and survival was available. Out of 51 biopsies which gave satisfactory immunocytochemical staining reactions, *bcl-2* protein was detectable in 39 (76%) cases. Expression of the protein was more frequent among the 29 low grade cases (85%) than in the 17 high grade cases (58%). In a substantial number of cases the pattern of protein expression was heterogeneous, some neoplastic cells expressing the protein and others being clearly negative. Since it has been suggested previously by Yunis et al (1989) in a small series of cases that the t(14;18) is of prognostic significance in follicular lymphoma, we have made a preliminary comparison between the presence or absence of *bcl-2* protein expression and patient response to therapy, the results of which will be presented.

28 DIRECT SEQUENCE ANALYSIS OF 14q+ AND 18q- CHROMOSOME JUNCTIONS AT THE MBR AND MCR REVEALING CLUSTERING WITHIN THE MBR IN FOLLICULAR LYMPHOMA. F.E.Cotter,C.Price,J.Meerabux, E.Zucca* & B.D. Young. ICRF Department of Medical Oncology, St Bartholomew's Hospital,London EC1A 7BE. *Division of Oncology, Ospedale San Giovanni, 6500Bellinzona, Switzerland.

The t(14;18) translocation is a highly consistent feature of follicular lymphoma although the underlying mechanism generating this fusion remains uncertain. The breakpoints on chromosome 18 at one of two sites, designated mbr and mcr, in the *bcl-2* gene. A PCR strategy has been developed for amplification and direct sequencing of the resultant 14q+ and 18q- reciprocal junctions. Sequence analysis of the amplified 14q+ junction established that 24 tumours contained a *bcl-2* (mbr) sequence fused to an immunoglobulin J_H region the majority being J₅ or J₆. A non-random pattern of breakpoints within the mbr region was found. Clustering of the breakpoint occurred with 8 translocating at the same base, while a further 8 had breakpoints within 10 bases. There was a second cluster within the mbr 50 bases 3' of the first cluster. One of these junctions had an unusual configuration with the *bcl-2* and J_H sequences separated by a recognisable D_H region. This suggests that at least some of the junctional sequences, previously thought of as N insertions, may be fragments of unrecognised D_H regions. In one of these tumours it was possible to sequence the reciprocal 18q- junction, showing it to consist of a D_H/*bcl-2* (mbr) fusion. Analysis of both reciprocal junctions for a translocation in the mcr region of *bcl-2*, showed that this 18q- junction also consisted of D_H fused to *bcl-2* sequence. In contrast to previous analyses, which demonstrated either loss or duplication of *bcl-2* sequences at the breakpoints, *bcl-2* sequence was conserved during the mbr and mcr translocations in this study. Although the precise mechanism of the t(14;18) translocation remains unclear, this data demonstrates that breakpoints are not randomly distributed within the mbr.

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29 HETEROGENEITY OF AGGRESSIVE LYMPHOMAS BY PLOIDY AND PROLIFERATIVE ACTIVITY. MORPHOLOGIC AND PROGNOSTIC IMPLICATIONS. P. Felman, C. Souchier, B. Coiffier, F. Berger, O. Gentilhomme, P.A. Bryon. Laboratory of analytical cytology, 69373 Lyon, and Haematology laboratory, Centre Hospitalier Lyon-Sud, 69310 Pierre-Bénite, France.

DNA analysis in malignant lymphomas has two purposes: a better understanding of their biologic behavior, and an improved refinement of prognostic subgroups. The aims of our study were: (1) to study proliferative activity and ploidy in large cell lymphomas, (2) to search for morphologic and prognostic correlations. DNA analysis was performed by image cytometry in order to measure lymphoma cells only, selected by the same morphologic criteria that are used for morphologic diagnosis.

Lymphomas (68 cases) were classified according to the working formulation by histology and cytology, including 8 D-M, 7 F-L, 36 D-L, 17 IB. In addition, each of both D-L and IB subgroups were subdivided into typical (monomorphic) and polymorphous types. Sixty two cases were studied at diagnosis, while six were transformed lymphomas. There were 59 B-cell and 6 T-cell derived tumors. Median age was 62 yrs. According to the Ann Arbor staging, 36% were stage I+II, 64% stage III+IV. 54% had a tumoral mass > 10 cm, 34% a bone marrow involvement and 55% an increased LDH level. The densitometric study was made with a Samba image processor, on Feulgen-stained touch imprints. Nuclear area, chromatin condensation and distribution, DNA content (mean and mode), and the proportion of cells in S and G2M phase were studied to characterize and compare the cases.

The mean proliferative activity increased from D-M (15.6%) to IB (28.5%) with a significant difference between D-M and F-L on one hand, D-L and IB on the other. Aneuploidy was observed in 44% of the whole group, with a clustering in the tetraploid region, corresponding mainly to D-L and IB cases. Polymorphous lymphomas of both subgroups looked very similar one from another, as regards either nuclear area, proliferative activity or the rate of tetraploidy. Transformed lymphomas had the same incidence of tetraploidy than the de novo cases.

Tetraploid lymphomas (19 cases), when compared to diploid or other aneuploid cases of the same histologic group, were characterized by a larger size, a more condensed and more irregularly distributed chromatin, and an unexpected lower proliferative activity ($p < .01$). 15 bore B markers, 3 were T-cell derived, 1 had no marker. Only 2 showed Ki-1 reactivity, without morphologic specific features. No clinical parameter (stage, tumor size, LDH level, CR rate) was related to the ploidy status, with the exception of age, tetraploid lymphomas being younger ($p < .05$). Tetraploidy had a favorable effect on overall survival, as had related features such as high values of nuclear size and low values of S phase; however, the Cox multivariate model identified clinical parameters only as strong prognostic factors.

Finally, our study underlines the close relationship between polymorphous subtypes of D-L and IB subgroups, as indicated by the absence of precise morphologic boundaries, and pinpoints the frequency (previously underestimated), and the characteristic features of tri/tetraploid lymphomas, within the heterogeneous group of large cell lymphomas.

30 α , β AND γ δ T-CELL RECEPTORS (TCR) IN PERIPHERAL T-CELL LYMPHOMA (P.T.C.L). Ph. Gaulard, P. Kanevaros, P. Bourquelot, C. Heloun, M. Divine, E.S. Zafrani, J.P. Farcat, F. Reyes. CHU Henri Mondor 94010 CRETEIL, France.

57 cases of PTCL were studied for cell expression of TCR chains, using monoclonal antibodies for the $\alpha\beta$ (BF1) and $\gamma\delta$ (anti-TCR δ -1) TCR. 51 cases were histologically classified according to the Working Formulation (mostly of the diffuse mixed and large cell subgroups) and to the Kiel updated classification; the 6 remaining cases were of the monomorphic medium sized type. Three phenotypic patterns were demonstrated. In 39 cases (69%) the phenotype (CD3+ BF1+TCR δ -1-) was that of most normal T cells expressing TCR- $\alpha\beta$. Most of these cases expressed either CD4 or CD8, lacked expression of at least one of the CD2, CD5, CD7 pan-T antigens and displayed a clonal rearrangement of the TCR- β locus. A second phenotypic pattern was found in 6 cases (10%), which were CD3+ BF1-TCR δ -1+, i.e. were the neoplastic counterpart of normal T cells expressing TCR- $\gamma\delta$. These cases were CD4-/CD8- and also lacked CD5 and CD7 pan-T antigens; 4 expressed the NK-associated CD56 molecule; they displayed clonal rearrangements of the TCR- δ locus. It is of interest that these $\gamma\delta$ lymphomas had unusual presentations since one case corresponded to a lethal midline granuloma and five to hepatosplenic tumors with a sinusoidal/sinusoidal infiltration. These five cases were classified as being of monomorphic medium sized type. The fact that the distribution of malignant $\gamma\delta$ cells in the splenic red pulp resembles that of normal $\gamma\delta$ cells reinforces the concept of a preferential homing of $\gamma\delta$ -T cells to this tissue. A third pattern (CD3+/- BF1-TCR δ -1-) was seen in 12 cases (21%) in which no evidence of either $\alpha\beta$ or $\gamma\delta$ TCR was found. 6 cases expressed CD3. In the 6 CD3- cases, a T cell origin was deduced on the basis of lack of B cell and myeloid antigens, expression of CD2, CD4 and clonal rearrangements of the TCR- γ , δ and β chain genes.

In summary, most PTCL are composed of cells which are related to the $\alpha\beta$ or less often $\gamma\delta$ -T cell subpopulations; however, in a significant proportion of cases, malignant cells fail to express either $\alpha\beta$ or $\gamma\delta$ TCR. The relationship between the latter cases and those expressing either TCR will be discussed.

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- 31** Ki67 AND 4F2 ANTIGEN EXPRESSION AS WELL AS DNA SYNTHESIS PREDICT SURVIVAL AT RELAPSE/TUMOUR PROGRESSION IN LOW-GRADE B-CELL LYMPHOMA. H. Holte, C. deLange Davies, K. Beiske, T. Stokke, P.F. Marton, E.B. Smeland, J. Høie, and S. Kvaløy. Institute for Cancer Research and The Norwegian Radium Hospital, Oslo, Norway

Previous work has shown that parameters of cell activation studied on lymphoma biopsies can be used to discriminate between low-grade and high-grade non-Hodgkin's lymphomas and to predict prognosis in the low-grade malignancy group only. We have now examined expression of several activation antigens and indicators of DNA-synthesis in 29 patients with low-grade malignant B-cell lymphomas at the time of primary diagnosis and later at relapse and/or tumour progression. At both times, the level of 4F2 antigen expression examined by flow cytometry on cells in suspension as well as the number of Ki67 antigen positive cells examined by immunohistochemistry were predictive of patient survival. DNA synthesis estimated by ³H-thymidine incorporation was of prognostic value at the second biopsy only. These parameters were more sensitive than histological demonstration of morphological transformation in secondary high-grade lymphomas in identifying high-risk patients at repeated biopsy. We propose that Ki67 or 4F2 expression or a marker of DNA synthesis (such as ³H-thymidine incorporation or labelling index) should be evaluated when repeated biopsies are performed, in order to select patients for whom aggressive chemotherapy may be considered.

- 32** HODGKIN'S DISEASE IN CHILDREN: TREATMENT REDUCTION BY ELIMINATION OF PROCARBAZINE FROM OP/PA/COPP CHEMOTHERAPY. RESULTS OF THE GERMAN COOPERATIVE STUDY DAL-HD-85. G. Schellong, I. Hörnig-Franz, J. Brämswig, M. Wannemacher (for the German DAL Hodgkin's Disease Study Group) Universitäts-Kinderklinik, D-4400 Münster and Radiologische Universitätsklinik, D-6900 Heidelberg, FRG

It was the objective of the DAL study HD-85 to reduce chemotherapy in comparison with study HD-82. In this preceding study with a total of 203 patients combined treatment strategy for all stages resulted in a relapse rate of only 3.5 % after 7 years, and an incidence of intercurrent deaths of 1.5 %. In study HD-85 procarbazine was eliminated from OP/PA (resulting in OPA) and replaced by methotrexate in COPP (resulting in COMP) to avoid the gonadotoxic long term effect in males. Chemotherapy consisted of 2 cycles of OPA for stages I/IIA (group 1), 2 cycles of OPA plus 2 cycles of COMP for stages IIB/IIIA (group 2), and 2 cycles of OPA plus 4 cycles of COMP for stages IIIB/IV (group 3). Subsequent radiotherapy was limited to the initially involved fields with doses of 35, 30 or 25 Gy depending on the extent of chemotherapy. Patients of groups 2 and 3 received 5 Gy to areas with incomplete tumor regression after chemotherapy.

Results: Between Jan. 1985 and Nov. 1986, 103 children below the age of 16 were enrolled in this study. 98 patients were treated according to protocol (59 boys, 39 girls). On the basis of a previously developed strategy for selective laparotomy and splenectomy 59/98 pts. (60 %) were laparotomized, but only 31/98 pts. (32 %) splenectomized. 4 patients showed progression of their disease under chemotherapy, all others achieved remission. 23 patients relapsed before Oct. 31, 1989. Due to an effective salvage therapy, only 1 child died. Thus, the 4 year survival rate in the total group is 99 %. The 4 year event-free survival is 72 % ± 2 % in the total group, 86 % ± 5 % in group 1 (n = 53), 57 % ± 11 % in group 2 (n = 21), and 54 % ± 10 % in group 3 (n = 24). These results are significantly poorer than in the preceding study HD-82: The 4 year event-free survival rates in this trial were 96 % ± 1 % in the total group (n = 203), 99 % ± 1 % in group 1 (n = 100), 96 % ± 3 % in group 2 (n = 53) and 90 % ± 4 % in group 3 (n = 50).

Conclusions: The elimination of procarbazine from the OP/PA/COPP chemotherapy, with all other conditions of therapy remaining unchanged resulted in markedly poorer treatment results as compared to the preceding study HD-82. These results prove that procarbazine is a very effective drug in Hodgkin's disease. Methotrexate at the applied dosage (2 x 40 mg/m² per COMP cycle) is not an adequate replacement in patients with stages IIB - IV.

33 HIGH-DOSE COMBINATION CHEMOTHERAPY FOR CHILDHOOD HODGKIN'S DISEASE: The French Pediatric Oncology Society experience. E.Bessa, O.Oberlin, O.Hartman, J.Michon, J.Bordignon, P.Hervé, F.Demeocq, D.Plantaz, T.Philip, G.Michel, A.Baruchel, G.Leverger.

The prognosis of patients with advanced Hodgkin's disease who fail first-line therapy or who relapse remains poor after conventional chemotherapy (CT). It has been shown that high-dose chemotherapy (HDCT) with autologous bone marrow transplantation can produce a high response rate in adults with advanced relapsed Hodgkin's disease. We report the French pediatric experience in 22 children with refractory or relapsed Hodgkin's disease who were treated between 10.84 and 10.88.

The median age at diagnosis was 13 years (range, 5-17 years). There were 17 boys. The median time from diagnosis to transplantation was 29 months (range, 10-103 months). All the patients had received MOPP regimen and 21 had also received a doxorubicin-containing program. Fifteen children had received a third line conventional CT with alternative drugs (mean 1.5 different cycles). All but 2 had received radiation therapy for the initial treatment.

At the time of relapse, 12 pts had nodal disease only, 3 had extranodal disease only and 7 had combined nodal and extranodal disease. At the time of HDCT, sixteen patients were responding to last chemotherapy including 6 who achieved complete remission (CR), 6 were progressive, failing to last chemotherapy attempt.

Conditioning regimens were C.B.V for 14 pts (BCNU, CPM, VP16), B.E.A.M. for 1 pt (BCNU, VP16, AraC, Melphalan), C.V.C for 4 pts (CCNU, CPM, VP16), C.B.V + AraC for 1 pt, C.V.C + AraC for 1 pt and CCNU + CPM + Vindesin + AraC for 1 pt. Three pts received also a total body irradiation (T.B.I.). All pts but one underwent autologous bone marrow transplantation; the last one received peripheral blood stem cells. In 2 pts with bone marrow involvement at the time of the relapse, in vitro purge was performed.

The estimated probability of survival is 67 % at 24 months. Fifteen pts are surviving, 4 of them with disease. The median time of survival is 26 months. Nine relapses occurred with a median delay of 6 months (range 3 - 31 months). The estimated probability of disease-free survival is 64 % at 24 months.

There were three therapy-related deaths and these pts are not evaluable for response. Taking in account the three toxic deaths as failure to therapy, the probability of event-free survival is 58 % at 24 months. Among the three toxic deaths, 2 were related to pneumonias, both in heavily previously irradiated patients: 1 had 20 grays to mediastinum before T.B.I. and the other one was treated when in forth relapse after repeated radiation therapy to mediastinum. One patient died from HIV infection after blood product contamination in 1984. These toxic deaths could be avoided now.

Even in this small series, status of the patients before HDCT appears predictive on outcome: among the 13 evaluable pts in partial remission or progressive disease before ABMT, only 4 remain in complete remission; by contrast all the six patients treated in complete remission still remain in continuous remission.

34 MALIGNANT HISTIOCYTOSIS AND LARGE CELL ANAPLASTIC (KI-1) LYMPHOMA IN CHILDREN AND ADOLESCENTS - PRELIMINARY EXPERIENCES OF THE BFM STUDY GROUP. P. Bucsky, A.C. Feller, A. Reiter, A. Heitger, H. Gadner, H. Riehm. Children's Hospital, Medical School Hannover, D-3000 Hannover, West-Germany

Malignant histiocytosis (MH) is considered as one of the neoplastic disorders of the mononuclear phagocytic system. Large cell anaplastic (Ki-1) lymphoma (LCAL) is a recently described non-Hodgkin's lymphoma entity. It was first recognized by its expression of the activation antigen CD30 and on the basis of a rather typical morphology. Previously, LCAL was diagnosed either as metastatic carcinoma, amelanotic melanoma, diffuse large cell lymphoma or as MH. Since true MH seems to be a rare disease in childhood and both MH and LCAL display some clinicopathological similarities, we reevaluated the clinical and morphological data of eleven children initially diagnosed as MH and entered into the cooperative German-Austrian therapy study DAL-HX 83. The diagnosis of MH could not be confirmed in any of these patients; in all children LCAL could be diagnosed. 18 patients with LCAL entered therapy studies NHL-BFM 83 and 86. Eight children were not included in any therapy studies.

Formerly, CD30 antigen has been shown to be expressed by both activated T, B lymphocytes, and histiocytes (Hi). Disregarding morphology and CD30 expression, cells of LCAL do not have a common, obligatory phenotype. It can be supposed, that this tumor cell type does not have a normal counterpart in the physiological lymphoid differentiation pathway. Therefore, we investigated the lineage specificity of neoplastic cells in 23 of these 37 patients. In 12 cases the neoplastic cells were of T-lymphoid, in 10 of non-T/B/Hi and in one of histiocytic phenotype. LCAL with B-lymphoid phenotype was not found in these pediatric patient group. In one child a secondary LCAL could be diagnosed, being developed on the basis of a small cell peripheral T cell lymphoma.

As to the therapy results, 29 children received the B cell lymphoma therapy regimen of the BFM Study Group. To date, 20 patients are in first and two in second complete remission. Thus, this therapy strategy seems actually to be an attractive treatment for LCAL.

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35 ANAPLASTIC LARGE-CELL LYMPHOMA (KI-1+/CD30+) IN CHILDHOOD. V.Vecchi, S.Pileri*, R. Bunnelli, P.Rosito, A.Pession, R.Rondelli, A.Borghetti, G.Paolucci III Department of Pediatrics - University of Bologna *Hemolymphopathology Section - Institute of Hematology - University of Bologna

In 1985 Stein et al. described a large cell lymphoma consisting of activated lymphoid elements, expressing the Reed-Sternberg cell - associated antigen Ki-1 (CD 30); this tumor has recently been enclosed in the updated Kiel classification and has been termed anaplastic large cell lymphoma (ALC).

Histologically, it is made up by of large neoplastic cells which tend to invade preferentially sinusoids and T-cell areas of the lymph node and which often show an apparently cohesive growth pattern. Many cases of this type of lymphoma have initially been diagnosed, because of their bizarre morphological features, as "malignant histiocytosis" or even as anaplastic carcinoma. In addition to Ki-1 antigen, lymphomatous cells often carry the interleukin-2 receptor (CD 25).

Many of these lymphomas express T associated antigens. Less commonly they appear to be of B cell origin, while a small group cannot be attributed to either B- or T- cell lineage. Genotypic analysis has supported these immunophenotypic results. Cytogenetic abnormalities have also been described: there is some evidence of a translocation involving the same region of chromosome 5 (q 35) and chromosomes 2 or 3.

From January 1983 to December 1989, 78 consecutive patients (pts) with non-Hodgkin's lymphoma (NHL) were admitted to the III Department of Pediatrics of Bologna University. Thirteen (16.6%) suffered from ALC. 7 pts were males, 6 were females; median age was 9 years (range: 3-12 years). At presentation, the commonest primary site of the tumor was a peripheral node (6 pts), followed by the subcutis (3 pts), mediastinum (2 pts), stomach (1 pt), and lung (1 pt). Besides the primary site, other localizations (such as bone lesions) were often detected during staging procedures. Based on the St. Jude system, 1 pt had stage I (8%), 7 pts stage II (54%) and 5 pts stage III (38%). 6 pts had fever and 1 of them arthritis. Neither weight loss nor night sweats were present in our pts. LDH serum levels were elevated in 4 out of 13 pts.

Histologic diagnosis of ALC was performed in all cases. 8 pts had T-ALC, common type, 1 pt had T-ALC, Hodgkin related type, 2 pts had T-ALC, macrophage rich type, and 2 pts had B-ALC common type. Morphological and immunohistological analysis was performed in all cases on formal-fixed and paraffin-embedded specimens. In 10 cases, cryopreserved material was also available and was employed for extensive immunophenotyping in frozen sections. T-cell receptor beta-gene rearrangement was demonstrated in both pts with T ALC, macrophage rich type; J chain gene rearrangement was observed in one pt with B-ALC.

Treatment modality All but one pts received chemotherapy according to a modified version of the LSA2-L2 protocol. The remaining pt was initially treated with a protocol for B NHL, because of the diagnosis of B-NHL, Burkitt type performed at another hospital. This pt was not considered evaluable for analysis for this reason. Local radiotherapy (RT) (20 Gy) was delivered on bulky lesions in 9/12 pts; the remaining pts did not receive local RT because of little (<5cm) subcutaneous or nodal involvement. Total duration of treatment was 2 years for all stages.

Results All 12 evaluable pts (100%) achieved complete remission (CR) after induction phase. 2 pts who did not receive local RT had local recurrence in the primary site; 1 of them showed also diffuse spread of the disease: CNS and multiple subcutaneous involvement. The Survival (SUR) and Event Free Survival (EFS) at 5 years, after a median observation time of 26 months, are 100% and 73% respectively. At present, 5 pts are off therapy in I CR, 1 pt is off therapy in II CR. The remaining 6 pts are on therapy: 5 in I CR, and 1 in II CR. Comparing these pts to all other pts with stage I-III T-cell NHL, treated with the same chemoradiotherapeutic regimen, we observed that the outcome is worse: in fact, T-cell NHL other than ALC showed 100% of EFS at 5 years.

In conclusion, ALC represents a peculiar entity from the histologic, biological and clinical points of view; extralymphatic involvement and systemic symptoms occur at high frequency. Considering the small and controversial data about the treatment program reported in the literature for this subtype of NHL, it appears that the best chemoradiotherapeutic protocol has not yet been established. However, our experience suggests that, while the immunohistologic profile should address the choice of chemotherapy, RT plays an important role in the local control of the disease.

36 PROLONGED DISEASE FREE SURVIVAL IN PEDIATRIC NON-HODGKIN'S LYMPHOMA USING IFOSFAMIDE CONTAINING COMBINATION CHEMOTHERAPY. N.Gad-El-Mawla, M.R.Hamza, S.Abdel-Hadi, M.H.Hussein, O.El-Tannir, I.Magrath. National Cancer Institute, Cairo, Egypt., and National Cancer Institute, Bethesda, U.S.A.

Pediatric non-Hodgkin's lymphoma (NHL) represents 16% of malignancies in children reporting to the National Cancer Institute, Cairo. The adopted treatment for these cases was the St. Jude's regimen consisting of: vincristine, cyclophosphamide, adriamycin, prednisone, and intrathecal methotrexate for the first 6 weeks for induction. Cranial irradiation followed for cranial prophylaxis. Once patient attains complete remission (CR), maintenance therapy follows for 3 years; methotrexate and purinethol by mouth. In 32 patients CR was achieved in 24; 75%, PR in one; 3%, 5 patients showed no response; 15%, while 2 patients died during the induction phase. At 60+ months follow up, 60% of cases are still alive, disease free. A new protocol was adopted in 1985 using a new combination. It consists of 2 alternating cycles: A, and B, for 4 - 8 cycles. Cycle A: cyclophosphamide, high dose Ara C, adriamycin, and vincristine. Cycle B: ifosfamide, methotrexate, and VP 16, with intrathecal methotrexate. The responses in 39 cases of pediatric NHL were as follows: CR in 31 cases; 82%, PR in 4 cases; 10%, no response in 3 cases; 8%. Under follow up at 60+ months, CR is still maintained in all cases; 82%. So, this new combination is more effective, has a better disease free percentage survival, shorter time of therapy no cranial irradiation with its long term sequelae, no maintenance therapy with prolonged use of oral methotrexate with its deleterious effect on the liver functions. Toxicities of both regimens were mild and both were well tolerated by all children. It is now applied as the line of therapy in all our cases of pediatric NHL.

37 IFOSFAMIDE (IF) IN THE TREATMENT OF HIGH-GRADE, RECURRENT B CELL LYMPHOMAS: EXPERIENCE OF THE PEDIATRIC BRANCH, NATIONAL CANCER INSTITUTE. I.T. Magrath, M. Adde, J. Sandlund and V. Jain. National Cancer Institute, Bethesda, Maryland, USA

We have conducted phase II studies of IF alone, and IF in combination with other drugs, in young patients with recurrent, small non-cleaved cell lymphomas and large cell lymphomas (immunoblastic). In the first study, 13 multiply relapsed patients were treated with IF, 1200 mg per m² per day as a continuous iv infusion for 5 days. This relatively low dose was used since mesna was unavailable in the USA at the time. All patients had extensive, recurrent B cell lymphoma: 10 had bone marrow (BM) involvement at the time of relapse and 5 of these 10 patients also had CNS involvement. All had been heavily pretreated with cyclophosphamide (CTX), and 7 had received very high dose CTX (45mg per m² per day for 4 consecutive days) with other drugs. 2 patients had complete responses (CR), 2 partial responses (PR), and 2 objective responses (OR), defined as a >50% reduction in serum LDH level. 2 patients were inevaluable. Toxicity consisted primarily of hematuria and myelosuppression. There were no long term survivors. We concluded, from these results, that IF is an active agent in B cell, high grade lymphomas. In an attempt to improve upon these results, we designed a regimen to be used at the time of first relapse, consisting of IF, 1500 mg per m² per day for 5 days (continuous infusion), mesna uroprotection, 1500 mg per m² per day for 5 days (continuous infusion), VP16, 60mg per m² per day for 5 days and ara-C, 2 gm per m² every 12 hours for 4 days. We planned to give 5 additional cycles at somewhat reduced dosage to responding patients. 13 patients with extensive recurrent disease were entered into the study. 4 patients had BM involvement and 4, CNS disease (one of the 4 had both BM and CNS involvement). 4 patients achieved CR and 4, PR. There were 2 ORs and 2 NRs. One additional patient, still undergoing therapy, has so far achieved a PR. The predominant toxicity was severe myelosuppression accompanied by septicemia in all patients. The average duration of neutropenia was 17 days. 3 patients died as a result of infection. One patient, who also received craniospinal irradiation, developed quadraplegia. 2 patients remain alive, one free of disease at present, and the other still responding to therapy, but follow up is short in these two patients. While toxic, this protocol appeared to be an effective regimen for patients with very high risk, recurrent disease. We have, therefore, incorporated a modified version of this regimen into a protocol for patients presenting per primum with high risk B cell lymphomas.

38 BFM THERAPY STRATEGY AND RESULTS IN ADVANCED CHILDHOOD B-CELL NEOPLASIAS (STAGE-IV B-NHL; B-ALL). A.Reiter¹, S.Sauter¹, S.Müller-Wehrich², J.Küh³, H.Gadner⁴, H.Riehm¹. Depts. of Pediatrics, Hannover¹, Munich², Würzburg³, Vienna⁴, FRG/Austria

Stage IV B-NHL (bone marrow invasion <25 % B-lymphoblasts and/or CNS involvement) and B-ALL (>=25 % bone marrow B-lymphoblasts) constituted for 35 %, 25 % and 26 % of all B-cell neoplasias in trials ALL/NHL-BFM 81, ALL/NHL-BFM 83 and ALL/NHL-BFM 86, respectively. In trials ALL/NHL 81 and ALL/NHL 83 therapy consisted of two alternating therapy elements composed by cyclophosphamide (CY), intermediate dose (ID) methotrexate (MTX, 0.5 g/m², 24 h), adriamycin, Ara-C, VM-26, and corticosteroids. In trial ALL/NHL 86 ID-MTX was replaced by high dose (HD) MTX (5 g/m², 24 h). Doses of Ara-C and VM-26 were increased, CY was partially replaced by ifosfamide, and vincristine was added. Eight therapy courses were given in trial ALL-BFM 81, six in trials ALL-BFM 83 and 86. In trial ALL-BFM 81 the dose of cranial radiation (RT) was 24 Gy for prevention and 30 Gy for overt disease. In trial ALL-BFM 83 local CNS chemotherapy was intensified with MTX/Ara-C via Omayra reservoir. After introduction of HD-MTX and triple drug intrathecal therapy in trial ALL/NHL-BFM 86, no preventive CNS RT and/or Omayra reservoirs were any longer used. Results are as follows:

BFM-trial	n	CNS pos	CR	NR	Relapses all CNS	Therapy death	pEFS
81	27	11	25	2	12 6	2	0.41
83	35	11	33	1	12 1	1	0.60
86	45	3	40	3	2 1	3	0.81

In trial ALL/NHL 81 CNS was the most frequent site of failure, whereas only one isolated CNS relapse occurred in trial ALL/NHL 83. Except for one all relapses took place within 12 months. In trial ALL/NHL 86 the probability of event free survival (pEFS) is 0.81 at 2 years (median observation time 21 months). Of three pts with initial CNS disease two received RT and failed (CNS nonresponse, CNS recurrence). Thus, HD-MTX proved to be highly effective for both, systemic and CNS failures. It is still unsettled, however, if HD-MTX is a highly effective element for prevention of CNS failure in pts with overt disease at diagnosis, since our experience in trial ALL/NHL 86 is rather limited. Regarding the different relapse patterns in all trials, we believe fractionated local CNS chemotherapy via Omayra reservoir to be more beneficial for pts with initial CNS disease, whereas RT seems to be ineffective.

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39 HIGH SURVIVAL RATE OF B-CELL NON HODGKIN'S LYMPHOMAS (B-NHL) WITH CNS INVOLVEMENT (CNS+) AND B-ALL. Results of the LMB 86 protocol of the FRENCH PEDIATRIC ONCOLOGY SOCIETY (SFOP). C.Patte, Y.Pere, G.Leverger, H.Ruble, J.Otten, D.Plantaz, M.Munzer, P.Boutard, E. Benz-Lemoine, B.Pautard, X. Rialland, C. Kalifa, for the SFOP

In the 1st study of the SFOP (J Clin Oncol 4 : 1219, 1986), event free survival (EFS) of the patients (pts) with B-NHL CNS+ was only 19%. For these pts and the pts with B-ALL (without abdominal or head and neck primaries), the LMB 86 protocol was initiated in October 1985.

A low dose chemotherapy (vincristine (VCR) 1 mg/m², cyclophosphamide (CPM) 300 mg/m², prednisone (pred) 60 mg/m² and triple intra-thecal injections (TIT) was administered 7 days (D) before the beginning of induction. The 2 induction courses combined high dose (HD) CPM (1.5 g/m² then 3 g/m²) and HD methotrexate (MTX)(8g/m² in 4 hr infusion) with adriamycin, VCR, pred and TIT. The 2 consolidation courses consisted in Ara-C 50 mg/m² in 12hr infusion D 1 to 5, followed by a push of 3 g/m² over 3 hr D2 to 5, and VP16 200 mg/m² D2 to 5. They were followed by 4 less intensive maintenance courses and a 24 Gy cranial irradiation was performed after the 1st maintenance course. None of the pts reported here received HD polychemotherapy with bone marrow (BM) transplantation in 1st complete remission (CR).

Between October 1985 and January 1989, 34 pts from 14 centers, were registered: 23 CNS+, 17 of them with BM involvement, and 11 B-ALL CNS-. There were 25 boys and 9 girls. Median age was 8 years (range 2-15 years). 3 pts died early: 1 before induction from aspergillosis and 2 during induction course aplasia. All other pts achieved CR. 2 pts CNS- relapsed in BM and 3 pts CNS+ relapsed in CNS ± BM within the 9 1st months. 26 pts are alive in 1st CR with a follow-up ≥ 1 year and can be considered as cured. The EFS is 76% (SD : 7) for all the pts, 72% (SD : 9) for the 23 CNS+ pts and 82% (SD : 12) for the 11 B-ALL CNS- pts.

In conclusion, it is now possible to obtain a high survival rate in pts with B-NHL CNS+ or B-ALL with a polychemotherapy regimen which does not include HD polychemotherapy with BMT.

40 RESULTS OF TREATMENT FOR ADVANCED STAGE (IV) DIFFUSE SMALL NON-CLEAVED CELL NON-HODGKINS (NHL) LYMPHOMAS AND B(SIG+) CELL ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): THE PEDIATRIC ONCOLOGY GROUP (POG) EXPERIENCE, 1986-89. WP Bowman, J Shuster, B Cook, F Behm, C Berard, and SB Murphy, on behalf of the Pediatric Oncology Group, St. Louis, Missouri, USA.

With the aim of improving the complete remission (CR) rate and event-free survival (EFS) for patients with advanced stage B-NHL and B-ALL, POG investigators devised a treatment plan, modified from the Total Therapy B protocol (J Clin Oncol 4:1732-1739, 1986), consisting of alternating courses of fractionated cyclophosphamide (300 mg/m² IV q12h x6) followed immediately by adriamycin (50 mg/m² IV) and vincristine (1.5 mg/m² IV) combined with intrathecal methotrexate and cytarabine, followed after hematopoietic recovery by sequential high-dose methotrexate (1 gm/m² IV) and high-dose cytarabine (3 gm/m² IV infusions q12h x4). Treatment courses are alternated and each repeated a total of four times, with the median duration of treatment extending 6-8 months. From October 1986 until August 1989, 30 B-ALLs and 21 stage IV NHLs had been accrued. The CR rate, assessed after 1 cycle each of the above-described treatments, is 80% for B-ALL and 93% for stage IV NHLs. The off-therapy EFS, assessed at 18 months, is 51% (± 16% SE) for B-ALL and 78% (± 21% SE) for stage IV B-NHL. Toxicity consisted of repeated profound myelosuppression, stomatitis, esophagitis, neurotoxicity (quadraparesis), bacteremias, disseminated fungal infections (4 patients) and 2 deaths in remission. Compared to the previous POG protocol (8106) for these patient groups, which resulted in longterm survival of 20% of subjects, these results represent a significant improvement.

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- 41** EPIDEMIOLOGICAL, PATHOLOGICAL, SEROLOGICAL AND CLINICAL STUDIES OF MALIGNANT LYMPHOMAS IN CHINA. Y. Sun, S.Z. Sung, Y.W. Li et al. Cancer Institute & Hospital, Chinese Academy of Medical Sciences, Beijing, China

During the past two decades, some special epidemiological and clinical features of malignant lymphomas (ML) in China have been noticed. Extensive studies were carried out in the recent years. On a nationwide retrograde survey, the age-adjusted mortality rate was 1.16 per 100,000, composed 1.73% and ranked 11 among all malignancies. The geographic distribution of ML showed that the mortality rate varied from 0.75 to 2.43, which was higher in large cities and along the coast, and lower in the inner provinces. No high-incidence area was found. The peak of age was 31-40 for Hodgkin's disease (HD), and 21-30 for non-Hodgkin's lymphoma (NHL). The proportion of HD was much lower than that in Western countries, but similar to Japan and India. The distribution of Nasopharyngeal carcinoma was different from that of ML. In a review of slides of 3366 cases of ML diagnosed during 1978-1982 as compiled from 12 different areas, the average figure for HD was 10.9%; while that of NHL was 89.1%. Among NHL cases, B-cell type composed of 67.9%, and only 4.4% of them was nodular; while T-cell type composed of 26.1%, and 6% unclassified. Using the ABC technique with various monoclonal and polyclonal antibodies, immunohistologic studies of 209 ML cases collected from 4 areas with relatively higher percentage of T-cell type NHL, confirmed that the figures of HD was 12.0%, and NHL 88.0%. T-cell type composed 33.9% of all NHL; while B-cell type was 62.4%, and non T non B type 3.7%. But typical cutaneous ATL was rare in our series. In order to reveal the possible relationship between T-cell lymphoma in China and T-cell leukemia/lymphoma virus (HTLV), sera samples from 427 ML patients, 427 normal subjects and 106 other cancer patients were assayed for HTLV antibody by immunofluorescence method. There were only 5 with positive results, while all other patients and normal subjects were negative. The results from Fujian province also showed no positive HTLV-I antibody among the sera of 138 patients with leukemia, 16 with ML and 647 normal individuals. There are only 4 cases of ATL reported in Chinese literature up to now, and two of them were identified by Japanese virologists. Studies of EBV antibodies showed that it seems no correlation between EBV infection and the development of ML in China. According to a review of more than 3000 cases of ML consecutively seen during 1958-1988 in Cancer Hospital, CAMS: (1) more than 95% of our NHL cases were diffuse type; (2) only 5% of all NHL patients were low grade and more than 30% were high grade; (3) NHL primarily developed in Waldeyer's ring often with gastrointestinal involvement, particularly the stomach and small intestine; (4) the prognosis of lymphoblastic lymphoma/leukemia patients was very poor, while that of immunoblastic lymphoma was good, and that of mycosis fungoides and Burkitt was reasonable. In order to improve the clinical results of multimodality treatment in ML, factors influencing the long-term survival are also analysed and will be discussed further.

- 42** T-cell lymphoma in Japan. M. Shimoyama, Hematology-Oncology and Clinical Cancer Chemotherapy Division, National Cancer Center Hospital, Tokyo 104, Japan.

Proportion of T-lymphoma accounts for about 15% of non-Hodgkin's lymphoma in Western countries, while it accounts for about 50% in Japan. This trend is mainly due to high incidence of adult T-cell leukemia-lymphoma (ATL) that is clustered in southern Japan. T-cell lymphoma is heterogeneous and has distinctive prognostic factors and clinical features that have not been observed in B-cell lymphoma. Apart from cutaneous T-cell lymphoma and lymphoblastic T-cell lymphoma, ATL, immunoblastic lymphadenopathy (IBL)-like T-cell lymphoma, nasal T-cell lymphoma, cutaneous Ki-1 lymphoma and usual peripheral T-cell lymphoma have been reported as distinctive disease entity. I describe here recent progress of T-cell lymphoma research in Japan. 1. ATL: Most of ATL have been known to be closely associated with HTLV-I, but about 7% were HTLV-I negative ATL. A cox proportional hazard model analysis of 854 patients with ATL revealed that five factors, advanced performance status, high LDH, age of 40 or more, increased No. of involved lesions and hypercalcemia, were negatively associated with survival at $P < 0.01$. These prognostic factors can be used to construct a model to identify patients at low and high risks for shortened survival. ATL is still difficult to treat successfully with chemotherapy. Complete remission was achieved in about 40% of the patients by doxorubicin or etoposide containing combination regimens. Incidence of ATL from healthy HTLV-I carriers was calculated to be about 0.1% per year after 40 years of age. There is a long latent period since infection of HTLV-I. HTLV-I has not been known to have mutagenic or oncogenic activity. So, other mechanisms than HTLV-I may be involved in leukemogenesis of ATL. This idea is supported by the indirect but reliable evidences such as the presence of HTLV-I negative ATL and multi-step leukemogenesis hypothesis of ATL evidenced by clear fitness of Weibull distribution of onset ages of ATL. Approximate number of independent leukemogenic events in ATL is calculated to be five. These events may be somatic mutations. 2. IBL-like T-cell lymphoma: This disease is a distinct peripheral T-cell lymphoma proposed by us. Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) and AILD-like lymphoma is the same category of the disease as IBL-like T-cell lymphoma because of the same histologic feature and clonal rearrangement of T-cell receptor beta chain gene. 3. Prognostic factors of peripheral T-cell lymphoma (PTCL): A cox model analysis of 158 patients with PTCL revealed LDH, PS, total protein, and number of involved lesions were major prognostic factors for survival, but pathology and stage were not. However, chemotherapeutic response and prognosis of PTCL were not poor and almost the same as unfavorable B-cell lymphoma.

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43 NEW INSIGHTS INTO THE BIOLOGY OF GERMINAL CENTER DERIVED NON-HODKINS LYMPHOMA. L. Nadler, F. Schriever, A. Freedman. Dana Farber Cancer Institute, Boston, Massachusetts, USA.

Although the 14;18 translocation with rearrangement of the bcl-2 oncogene is seen in the majority of follicular lymphomas, this defect is not sufficient to explain the phenotypic (i.e. clinical) behavior of these tumors. Follicular lymphomas appear to be the neoplastic counterparts of the germinal center of the secondary follicle. The cellular components of the normal secondary lymphoid follicle are poorly understood. Therefore, we have attempted to isolate and study populations of germinal center derived cells as a first step to understanding the clinical behavior of these tumors.

The germinal center of the lymphoid follicle is a specialized microenvironment which plays a critical role in the proliferation and differentiation of B lymphocytes. The major constituent of germinal centers are activated B cells but follicular dendritic cells (FDC) as well as some macrophages and T cell subsets are also located there. Within the germinal center, activated B cells are physically associated with the FDC, but the precise functional interactions between B cells and other cellular components are unknown.

There is increasing evidence that most human B cell malignancies correspond to subpopulations of activated B cells. Activated B lymphocytes within the germinal center appear to both morphologically and phenotypically heterogeneous. Although immunohistochemical studies suggest that germinal center B cells express the CD10 (CALLA) antigen, to date, activated B cells isolated from normal human lymphoid tissues have been CD10 negative. Attempts to induce CD10 via a number of activation pathways have not convincingly induced this antigen and therefore the subpopulation of activated B cells which correspond to the small cleaved follicular lymphoma cell is still unknown.

Of great interest is the observation that virtually all follicular lymphomas have FDCs within the malignant germinal center. The relationship between the FDC and the clinical behavior of follicular lymphoma is presently unknown. However, we have recently isolated the FDC to homogeneity and have evidence that this cell is of a unique lineage. Isolated FDCs do not express a phenotype characteristic of a hematopoietic lineage cell (CD14+, CD19+, CD45-). The major cell surface antigens expressed on these cells are complement receptors (especially CD21) and adhesion molecules (VLAs and ICAM-1). More recently, single cell PCR examination of these cells provides confirmation that they express mRNA for the CD21 gene but not for the CD45 and CD20 genes. These cells do not express message for PDGF receptors, CD4, TNF- α , and IFN- γ genes providing additional evidence that they lack mRNAs specific for cells of haematopoietic and fibroblastic origin.

We have recently demonstrated that activated human B cells bind to the germinal center via a novel ligand-receptor interaction. A frozen section binding assay was developed to identify those molecules involved in adhesive interactions between human-B cells and lymphoid follicles. Activated human-B cells were found to selectively adhere to germinal centers. The VLA-4 molecule on the lymphocyte and the adhesion molecule INCAM-110 (VCAM) expressed on FDCs are, in part, responsible for this interaction. Although FDCs are clearly involved in the localization of B cells within the germinal center as well as presentation of antigen, it is unknown whether they have other important functions. It is possible that these cells provide signals via cell-cell contact or cytokines which are crucial to regulating the events of proliferation and differentiation of B cells. The above studies provide a system to examine the mechanisms of localization of neoplastic germinal center derived B cells. New systems will be required to examine the functional importance of the FDC-B cell interaction. These systems now permit the examination of the neoplastic B cells with its microenvironment.

44 The significance of B-clonal excess in peripheral blood in patients with non-Hodgkin's lymphoma in clinical complete remission.

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By taking advantage of the monoclonal nature of non-Hodgkin's lymphoma (NHL) it has been possible to detect small numbers of lymphoma cells which are not evident by routine morphological methods. These new methods are based on the detection of either a restricted light chain expression - clonal excess analysis (CE) or a monoclonal gene rearrangement of the Ig-chains or the T-cell receptor.

We have studied the impact on relapse and survival in NHL patients in clinical complete remission (CCR) with evidence of remaining tumour cells as detected by CE analysis of peripheral blood.

PATIENTS AND METHOD.

The study includes 232 patients with NHL, 43% low grade and 43% high grade malignant (Kiel classification). Patients with stage I disease (46%) had been treated with involved field radiotherapy and patients with stage II-IV disease with chemotherapy. Aggressive multi-agent chemotherapy (MEV, CHOP/M, MACOP-B) was used in high grade NHL and less intensive chemotherapy (COP, Stereocyt) in low grade NHL. The patients were off treatment and in CCR. At regular intervals routine hematology, microscopy and CE analysis were performed. The number of samples per patient were 1 - 10 (median 4). The kappa and lambda distributions were analysed by direct immunofluorescence in flow cytometry. CE was estimated by visually comparing the two distributions. A distinct incongruence was interpreted as a CE, indicating monoclonal cells i.e. lymphoma cells.

RESULTS.

CE was found in 28/232 (12%) and more frequently in low grade (18%) than in high grade malignant NHL (9%). In patients with morphological bone marrow involvement at initial staging CE occurred in 27% in CCR compared to 10% in those who had not. Despite this there was no correlation between initial stage and the occurrence of CE in CCR. Time to relapse and survival did not differ in patients with or without CE.

CONCLUSION.

It would seem logical to assume that tumour cells remaining after therapy would imply a clinical relapse. Despite a follow up of 0 - 531 (median 59) months of these patients in CCR, we could not confirm this assumption. Neither relapse free survival nor survival was confounded. The conclusion to be drawn is that an isolated finding of CE is of no prognostic significance.