THE CURE OF HODGKIN'S DISEASE: HALF A CENTURY OF REMARKABLE PROGRESS
Saul A. Rosenberg, Stanford, USA

It is now a reality that Hodgkin's Disease can be cured with initial treatment programs in virtually all patients, except the elderly. Histologic factors, staging methods and prognostic groups are becoming less and less relevant.

The current protocols used at Stanford and elsewhere will be reviewed to emphasize that combined modality is really the key to improving the cure rate and minimizing late complications.

The experience gained from treating over 2500 patients at a single institution will be used to support these conclusions.
Oral presentations

1. Special session on pathology

The Problem of Classifying Lymphomas—An Ongoing Challenge for Progress. B. Swartz-Cohen

In the late 1960s and early 1970s the most widely recognized "new" classifications of the non-Hodgkin lymphomas were those proposed by Rappaport (the "Rappaport classification") and by Lennert (the "Lennert classification"). With the advent of immunological and histological markers in the early 1970s, however, new concepts arose to supplement the purely morphologic approach to diagnosis and classification of these tumors. Lymphomas were increasingly recognized to be neoplasms of the immune system, composed of malignant proliferations which retained many of the morphologic and functional characteristics of their normal counterparts. These advances led to a flurry of new classifications proposed in 1974-75, leading to confusion for both clinicians and pathologists, perhaps most evident at the International Cancer Congress in Florence in 1974. To address this problem, the National Cancer Institute (USA) sponsored an international workshop of expert pathologists and clinicians on Sept. 4-5, 1975. It became apparent at that meeting that only a well-organized retrospective study would provide data for meaningful progress and resolution of differences. From 1976-1980 such a massive collaborative project was accomplished and served as the basis for the Working Formulation for Clinical Usage, proposed as a vehicle for translation among the six tested schema. Since the formulation was published in 1982 there have been numerous strides in scientific and clinical understanding of these cancers, fueled by contributions from immunology, genomics, and molecular biology. To recognize and disseminate understanding of these newer observations, an International Lymphoma Study Group promulgated in 1994 a new proposal entitled "A Revised European-American Classification of Lymphoid Neoplasms". As a sequel to another international assembly of pathologists and clinicians, held at the National Cancer Institute (USA) on March 21-23, 1994, a second large-scale retrospective study has been accomplished, the results of which are to be presented at this Conference, along with data from other international committees and cooperative groups. Concurrent with these events the World Health Organization has enlisted a committee of expert pathologists to prepare a new edition of "Non-Hodgkin Lymphomas: A Report of the Histological Subcommittees and a Clinical Advisory Committee", with broad international representation, this body should generate in the near future a consensus proposal with broad scientific and geographic support. These historical and ongoing efforts in lymphoma pathology are a paradigm for progress in clinicopathologic understanding of all cancers.

New Data on Molecular Biology for Classification
Harald Stein, Institute for Pathology, Klinikum Benjamin Franklin, Freie Universität, 12209 Berlin, Germany

There is accumulating evidence that the molecular pathogenesis of malignant lymphomas (MLs) is due to a clustering of genetic lesions leading to distinct molecular pathways. In most instances they represent intrahematopoietic translocations, each of which appear to be associated with a distinct lymphoma entity, e.g. mantle cell lymphoma with the (11;14), follicle centre lymphoma with the (14;18), Burkitt lymphoma with the (8;14), and anaplastic large cell lymphoma with the (2;5). The mentioned genetic lesions probably constitute primary events. Trisomies detected in B-CLL (trisomy 12), in extranodal marginal zone lymphomas (trisomy 3) and in other may also represent primary events. In contrast, the inactivation of suppressor genes are considered as secondary hits. They are either caused by mutations or by single gene inactivation. These events, of which the alternation of p53 gene is an example, are shared by more than one ML entity and they usually arise during progression of a disease into a higher malignant form, e.g. during the transformation of follicle centre lymphomas into diffuse large cell lymphomas. Other genetic lesions involve viral infections, some of which appear to be entity restricted, e.g. adult T-cell leukaemia/lymphomas with HTLV-I, body cavity lymphoma with HHV-8, while others share an infection with the same virus, e.g. Burkitt's lymphoma, Hodgkin's disease, certain peripheral T-cell lymphomas, plasmablastic lymphomas and HIV associated lymphomas with EBV. However, in the latter mentioned lymphomas, the EBV gene activation pattern differs in relation to their type. ML also differs in terms of somatic mutations in the variable immunoglobulin genes involved in clonal rearrangements. Since the mentioned genetic lesions are developmentally generated during the germinal centre reaction, it is possible to distinguish, based on the pattern of the immunoglobulin V gene mutations, B-cell lymphomas that are derived from preB-cell centers, B-cell germin centers or postgermin centers B-cells. The implications of all above mentioned genetic features for the classification of ML will be discussed.

THE REVISED EUROPEAN-AMERICAN LYMPHOMA CLASSIFICATION (1)

M. Lennert (for the International Lymphoma Study Group)

New information in the last decade has resulted in reorganization of lymphoid neoplasms and refinement of some previously recognized disease categories, together with international differences in terminology and definitions, this has caused confusion among practicing pathologists and clinicians. The ELN using published data and personal experience, developed a consensus list of currently recognizable lymphoid neoplasms that can be recognized with available morphologic, immunologic, and genetic techniques, and which have distinctive clinical features. Immunophenotyping and genetic features are important in defining neoplasms, but are not required for diagnosis in typical cases. We proposed uniform terminology and criteria for diagnosis. The resulting list (a revision of current European and American classifications) is proposed as interim lymphoma classification. Lymphoid neoplasms are categorized at B cell, T-cell and Hodgkin's disease. T and B-cell neoplasms are divided into precursor and peripheral types, and can be grouped by clinical aggressiveness (provisional).

B-CELL NEOPLASMS

- B-Cell CLL/PLL, PLL
- Lymphoplasmacytotic lymphoma
- hairy cell leukemia
- plasma cell myeloma
- Splenic marginal zone lymphoma
- Indolent extranodal lymphomas
- Extramedullary marginal zone lymphomas
- Mucosa associated lymphoid tissue
- Lymphoid nodal lymphomas
- nodal marginal zone lymphoma
- Follicle center lymphoma (follicular lymphoma) (grade 1-3+)
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
- Anaplastic large cell lymphoma
- Periocular T-cell lymphomas (alveolus*)

AGGRESSIVE (untrialled survival measured in months)

- Precursor B-cell lymphoblastic lymphoma
- Indolent lymphomas
- Burkitt's lymphoma
- T-cell lymphoblastic lymphoma
- Adult T-cell lymphoma (AIDS) (HIV+/-)

HODGKIN'S DISEASE

- Lymphocyte predominant, nodular, diffuse
- Classic Hodgkin's disease
- Mixed Cellularity
- Lymphocyte-depleted
- Lymphocyte-poor classical HD

- Anaplastic Large-Cell Lymphoma, Hodgkin's-Like subtype

APPLICATION OF THE INTERNATIONAL LYMPHOMA STUDY GROUP (ILSG) CLASSIFICATION OF NON-HODGKIN'S LYMPHOMA (NHL): CLINICAL CHARACTERISTICS AND OUTCOME OF 1400 PATIENTS FROM 8 COUNTRIES.

The International Non-Hodgkin's Lymphoma Classification Project. Omega, NE 61918-3330, USA

An international group of pathologists and internists collaborated to apply the recently published ILSG classification of NHL to 1400 NHL cases consecutively diagnosed at 8 institutions between 1988 and 1991. Five review pathologists and a designated institutional pathologist independently classified all cases by the Working Formulation, IEL and ILSG classifications. A random subset of cases from each site were re-classified by each review pathologist. Data on immunophenotype, clinical characteristics, treatment and follow-up data were supplied by the local institution. An interim analysis of the first 916 available cases showed the following distribution by consensus ILSG classification, and 4-year failure-free survival (FFS) and overall survival (OS) estimates (5 largest categories):
CLINICAL BEHAVIOR OF 2,168 PATIENTS TREATED ON SOUTHWEST ONCOLOGY GROUP (SWOG) PROTOCOLS AND RECLASSIFIED ACCORDING TO THE R.E.A.L. CLASSIFICATION

RI Fisher, TM Groagan, S Dahlberg, R Baziell, P Banks, B Nathwani, C Kjeldsberg, and TP Miller, Maywood, IL, USA

In order to determine the clinical characteristics and therapeutic relevance of the newly described R.E.A.L. lymphoma categories, we conducted a consensus-based morphologic review of 2,168 patients previously categorized by the Working Formulation (WF) and entered on prior SWOG studies (7204, 7426, 7713, 8410, 8505, 8516). All patients had bulky Stage II, III, or IV disease and received doxorubicin-based chemotherapy with curative intent. Follow-up ranged from 6-22 years. Of the new entities, median survival of monocyctoid B (MCBC) > mucosa-associated (MALT) > mantle cell (MCL); survival of anaplastic large cell (ALCL) did not differ from WF diffuse large cell (P = .32). Three major histologic risk groups could be constructed using both median survival (MS) (slope of survival curve) and therapeutic effect (shape of survival curve) (P = 0.0001): (1) low risk: MS = 86 mo; (2) intermediate risk: MS = 45 mo; (3) high risk: MS < 39 mo. Survival of both low and intermediate groups continued to decline over time, while the high risk group developed a plateau indicating potential for cure. The low risk group combines all follicular lymphomas and MCBC. The intermediate risk group combines R.E.A.L. categories of MCL and MALT with WF categories small lymphocytic (A) and diffuse small cleaved (E). High risk includes the ALCL with WF diffuse mixed (F), large cell (G & H) and small non-cleaved (J) lymphomas. The R.E.A.L. classification identifies important clinicopathologic entities. Clinical usefulness of the classification can be facilitated by combining categories based on similar survival curves following current therapy.

MOLECULAR BIOLOGY OF LYMPHOMAS ARISING IN MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT)

L.X. Pan and P.G. Isaacson. Department of Histopathology, University College London Medical School, University Street, London WC1E 6JJ

The indolent clinical course and reactive background of B-cell lymphomas of mucosa associated lymphoid tissue (MALT) often lead to controversy over their neoplastic and malignant nature. However, by analysing rearrangements of the immunoglobulin genes, we have been able to demonstrate monoclonality in the vast majority of the tumour cases collected over past ten years. In addition, we have obtained genetic evidence showing that the tumour clones are capable of disseminating to other organs, colonising follicle centres and transforming into high grade lymphomas. Although the molecular mechanisms for the pathogenesis of the tumours still remain largely unknown, MALT lymphomas rarely show rearrangements of proto-oncogenes, such as bcl-1 and bcl-2 involved in chromosome translocations, which are common in nodal lymphomas. Our recent studies have demonstrated that MALT lymphomas exhibit a high incidence of RER (recapitulation error) related genetic instability indicating a mutator phenotype which might be associated with chronic inflammation. In accordance with this, we have noted mutations of the p53 and c-myc genes in a significant proportion of MALT lymphomas. The accumulating abnormalities of the p53 gene including mutations, allele loss and overexpression have also been found to be associated with high grade transformation of the tumours. These genetic features together with other abnormalities, such as high incidence of trisomy 3 may form the molecular basis for evolution and progression of MALT lymphomas.

THE PATHOLOGY OF GASTRIC MALT LYMPHOMAS

Peter G Isaacson. Department of histopathology, UCL Medical School, London

The histopathological features of low grade primary gastric lymphomas recapitulate the structure of Peyer's patch (mucosa-associated lymphoid tissue) rather than those of lymph nodes. Transformation of low grade MALT lymphoma to high grade disease is well recognized and it is likely that most high grade primary gastric lymphomas evolve from low grade lymphoma of MALT type and are, therefore, derived from the same B-cell lineage. Molecular genetic studies of gastric MALT lymphomas have shown that MALT lymphomas do not share any of the features common to nodal lymphomas but, instead, have shown a marked increase in the frequency of trisomy 3. Gastric MALT lymphomas also differ from their nodal counterparts with respect to their clinical behaviour which is remarkably favourable.

The histological features of gastric MALT lymphoma suggest that one explanation for their favourable behaviour could be that their growth is influenced by antigen. That lymphoma should arise from gastric mucosa is paradoxical since there is no lymphoid tissue in the normal stomach. Several studies have, however, shown that lymphoid tissue accumulates in gastric mucosa almost exclusively as a consequence of H pylori infection and that this lymphoid tissue has MALT characteristics. These findings suggest that H pylori might provide the antigenic stimulus for the growth of gastric MALT lymphoma. Further evidence for this was the finding of H pylori in over 90% of cases of gastric MALT lymphoma. Subsequently, evidence supporting an antigenic role for the organism has steadily accumulated. The incidence of gastric lymphoma is higher in communities with a high prevalence of H pylori and a case control study has shown that gastric lymphoma is uncommon in patients infected with the organisms; moreover, the infection precedes the onset of lymphoma.

Laboratory studies have shown that the growth of tumour cells from low grade gastric lymphomas can be stimulated by H pylori and that the effect is strain specific and mediated by contact dependent help from H pylori specific T cells. Parallel clinical studies have shown that low grade gastric lymphomas, when confined to the mucosa, may regress following eradication of H pylori. It remains to be seen whether deeper penetrating or high grade tumours will respond in the same way.

TREATMENT OF GASTRIC MALT LYMPHOMA

F. Cavalli, E. Roggero and E. Zucca.
Servizio OncoLogicale Cantonale Ospedale San Giovanni Bellinzona, Switzerland.

The concept of lymphoma arising from the MALT has been widely accepted as an extranodal type of low-grade malignant entity, challenging the pre-existing concept of pseudolymphomas. There is increasing epidemiological and histopathological evidence of a plausible etiological relationship between gastric MALT lymphomas and H pylori infection, which is found in nearly all cases.

The clinical characteristics and the treatment outcome following different therapeutic approaches in a series of 93 patients (45 women and 47 men, median age 63 years) with localized (stage I) low grade MALT lymphoma of the stomach will be discussed. The patients have been treated with different modalities: the type of treatment depended on the policy followed at the time of diagnosis. Twenty-four patients underwent surgery (17 total and 7 partial gastrectomy): 13 had surgery alone and 11 received additional treatment, chemotherapy (9 cases) or radiation therapy (2 patients). Eleven patients had chemotherapy alone: 5 were treated with single agent chlorambucil, 5 in combination chemotherapy (4 with CHOP and one with CVP). Only one patient had radiotherapy alone. Forty-nine patients (all with stage I disease) had antibiotics against H pylori as their sole initial treatment. One patient had antibiotics and chlorambucil as initial therapy. Actuarial overall survival of patients in the series as a whole was approx. 85% at 10 years. At a median follow up time of 33.5 months, 10 of 83 patients have died and death was associated with second cancer in all cases with the exception of one patient who died of disseminated high-grade lymphoma after histologic transformation of the low-grade MALT lymphoma. The relationship between the length of overall survival and the presence of additional tumors was statistically significant (p<0.0001). Longer overall survival was also associated with better performance status (p<0.003). Both, performance status and second tumors were independently associated with survival in the multivariate analysis.

There was no significant difference in survival between patients who received different treatments (antibiotics, chemotherapy, surgery with and without adjuvant chemotherapy and/or radiotherapy). Surgery can be curative, however it carries a considerable risk of morbidity and mortality. Histologic and molecular regressions of gastric MALT lymphoma were achieved after eradication of H pylori in more than one half of patients who received antibiotic therapy, however, it is still unknown whether or not treatment for H pylori will definitely cure the lymphoma and prevent its relapse.

1. Special session on pathology
TREATMENT OF NON-GASTROINTESTINAL MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA PATIENTS.

B. Coiffier. Hospices Civils de Lyon, France.

If numerous papers describe the treatment of gastrointestinal MALT lymphoma patients, only anecdotal and retrospective studies summarize the treatment of non-gastrointestinal MALT lymphoma patients. Involved organs may be lung, thyroid, salivary glands, conjunctiva, breast, skin, urinary bladder, kidney, gall bladder, and thymus. These locations may be localized, associated with gastrointestinal involvement, or associated with disseminated lymphoma involving lymph nodes, spleen, or bone marrow. In retrospective studies, mostly localized locations were described and often treatment was surgery and/or localized radiotherapy. Results of this treatment was immediately good with obtention of a complete remission (CR) and no or few progressions during the following 3 or 4 years. Long term survival was rarely reported.

In our experience, 72% of the patients had stage IE or IIE, 24% stage IV, 15% bone marrow involvement. 73% of the patients responded to treatment with 66% CR. 33% of the patients progressed, 65% in the same organ and 35% in other organs. Response to treatment and survival was not different from that observed in gastrointestinal MALT lymphoma patients. Shortened disease-free survival and shortened survival were associated with impaired performance status, stage IV, spleen involvement, and anemia.

Patients with localized non-gastric MALT lymphoma may be treated with surgery, local radiotherapy, or chlorambucil. Currently, we prefer using chlorambucil (16 mg/m²/d x 5 days every month) for 6 to 12 months because of the long term problems observed with radiotherapy or of surgery sequelae. Patients with disseminated forms have to be treated with chemotherapy and fludarabine or chlorambucil has the same efficacy for obtaining CR. In patients with large tumoral mass or an important contingent of large cells, CHOP chemotherapy have to be used.
TREATMENT OF HODGKIN'S DISEASE (HD) IN CHILDREN WITH MOPP/ABVD WITHOUT RADIOThERAPY

H. van den Berg, J. Zeiss, H. Behrendt, Emma Kinderziekenhuis AMC, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

OBJECTIVES
Treatment of children with HD with non-cross-resistant drugs, without radiotherapy.

BACKGROUND
Since the introduction of MOPP, children with HD can be treated with chemotherapy alone. Side-effects of irradiation will be absent, but from alkylating agents in MOPP gonadal dysfunction and secondary malignancies are known. Combination chemotherapy in ABVD Might give better results and might decrease occurrence of side-effects.

DESIGN/METHODS
From 1988 to 1993 children with HD were treated with alternating MOPP and ABVD (3x MOPP, 3x ABVD).

RESULTS
21 children (7 F/13 M), age 5 to 18 years (median 14 years) were included. Clinical stages: I 7, II 8, III 5, IV 1 Pathology: 2 LP, 17 NS, 1 MC. In 1 patient only cytology was done. Two children relapsed. Toxicity: no decrease on cardiac ultrasound, no pulmonary effects by CO-diffusion, 6/10 had normal FSH and LH values, no thyroid dysfunction, no secondary tumors.

CONCLUSION
Children with HD can be treated without radiotherapy. Gonadal dysfunction seems less frequent than with MOPP. Prolonged follow-up is needed.

LONG-TERM PULMONARY SEQUELAE AFTER TREATMENT OF CHILDHOOD HODGKIN DISEASE (HD)

G. Bossi, L.Celesti, E.Volpi*, A.Corsico*, A.Baldasso**, F.Corrinella* and M.Racic* Department of Pediatrics, Institute of Respiratory Diseases*, Oncology Radiotherapy*, Policlinico San Matteo, Pavia, Italy

Pulmonary sequelae have been reported in patients treated for HD. Few data are available about patients treated for childhood HD followed over several years.

In a cross-sectional study carried on 76 months (median time) after radiotherapy we evaluated the lung function abnormalities and respiratory symptoms in 26 patients (15 males and 11 females) with HD diagnosed between 1983 and 1994 (median age at diagnosis 11 yrs, range 2-16 yrs). They had been treated with chemotherapy and radiotherapy according to protocol AIOP-MH 1983 (n=15) or AIOP-MH 1989 (n=11). At the time of the study 25 patients were in first complete remission and one in second remission. Of the 26 pts, 23 had spirometric impairment (dose 20Gy in 9 pts, 35Gy in 13 pts, and ≥6Gy in 3 yrs), and in 20/26 pts radiation field included the mediastinum. Forced vital capacity (FVC), functional residual capacity (FRC), forced expiratory volume in one second (FEV1), FEV1/FRC ratio, maximal expiratory flow at 25% of FVC were registered; diffusion capacity for carbon monoxide (DLco) was determined and corrected for hemoglobin content. Data were expressed as standard deviation score (SDS): actual result - predicted result / population SD and defined as pathological when <-1.64. Four patients of respiratory function abnormalities were defined: restrictive (FVC SDS -1.64 with FEV1/FVC index <80%), obstructive (FEV1-SDS 1.64 with FVC index 80%), isolated bronchial impairment (MBF SDS 1.64 with all other parameters normal), isolated diffusing impairment (DLco SDS 1.64 with all the other parameters normal). Oxygen saturation was measured by pulse oximetry. Reference values were taken from the literature (Kroeshaal, Thorax 1993).

Three pts referred dyspnea on exertion and 1 cough and phlegm; 19% of pts showed pathological FVC SDS and 31% showed pathological DLco SDS. The mean values were still in normal range although <10 yrs. A residual restrictive pattern was found in 19% of the subjects with restrictive pattern also had pathological DLco SDS. Another 19% had isolated diffusing impairment. Oxygen saturation was normal in all. Occurrence of lung abnormalities was not associated to age at irradiation and time elapsed from irradiation.

Adolescents and young adults treated for childhood HD are at risk for lung function abnormalities. Long-term follow-up should be offered to those patients because of their limited potential for pulmonary function recovery and possible lesser resistance to adverse agents as smoke, pollution, infections and aging.

CATIONIC LIPID DELIVERY OF C-MYC ANTISENSE OLIGONUCLEOTIDE TO RAPIDLY REDUCE VIABILITY, ProliferATION, AND C-MYC PROTEIN EXPRESSION IN TWO BURKITT LYMPHOMA (BL) CELL LINES: ANTI-CDDP AND NEEDED PCR TO MONITOR EX VIVO PURGING IN MIXED CULTURES

Williams, S. A., Herbst, T., Chang, L., Seib, T., Suen, Y., and Cairo, M. S. Children's Hospital of Orange County, CA 92668

Purification assays to determine the inhibitory effect of the AS were performed after a 5 hour incubation of cells in serum-free RPMI 1640 containing 8.5 µM LFM. An AS concentration of 3.54 nM showed maximal decrease in proliferation for both cell lines. D responded to LFM-AS complexes with a significant 15% decrease in proliferation, compared to cells which received no treatment or AS alone (p<0.0001), while those treated with LFM-AS showed an insignificant 4% decrease (p=0.26). ST showed a similar response to LFM-AS combination with a 23% increase in proliferation, as compared to no treatment or AS alone (p<0.003), while treatment with LFM-AS gave an insignificant 4% decrease (p=0.42). Controls, which included sense oligonucleotide, reverse AS, and AS without LFM, did not show a significant change from either cells receiving no treatment or LFM alone. Cationic assays of D and ST treated with its specific LFM AS revealed approximately 50% inhibition of colony formation after the 5 hour incubation. Flow cyrometry (FC) and immunoblot analysis showed a similar 2-fold reduction in intracellular c-myc protein levels. In contrast, incubation of normal human bone marrow with LFM-AS did not affect its cellular proliferation, viability or surface marker expression (CD4, CD8, CD19, and HLA-DR). Internalization of AS into D cells was studied using FITC-labeled 5'-AS. FC showed that only 2% of D treated with 5'-AS alone was associated with any fluorescent (FL) signal, while 65-73% of those incubated with LFM-AS had associated FL. FL from both groups were also analyzed by confocal microscopy to localize the FITC-labeled 5'-AS. FL was considerably more apparent in cells incubated with LFM-AS compared to 5'-AS alone and was distributed within the cell. FC with anti-cDDP and treated PCR were performed to monitor the extent of BL purging from mixed cultures. Anti-CDDP FC detected 0.5% BL in mixed cultures reliably. Using the more sensitive PCR assay with two nested sets of primers spanning the translocation, one tumor cell in 10 normal cells (0.001% tumor cells) was detected. This AS approach may form the basis for specific AB lipoid therapy, either alone or in a cocktail of other agents, in ex vivo molecule purging of autologous stem cell transplant for BL.
APPLICATION OF LONG PCR TO DETECT t(8;14)(q24;q32) TRANSLOCATIONS IN CHILDHOOD BURKITT'S LYMPHOMA AND B-ALL

U. zur Stadt, G. Hoser*, A. Reiter, K. Welte and K.W. Sykora, Department of Pediatric Hematology and Oncology, Hannover Medical School, 30625 Hannover, Germany and *Department of Cytopology, University Hospital, Warsaw, Poland

Burkitt's lymphoma (BL) and B-ALL are characterized by chromosomal translocations juxtaposing the c-myc gene on Chromosome 8 to one of the immunoglobulin loci. Translocations involving the immunoglobulin heavy chain (Igh) on chromosome 14 were found in approximately 75-90% of these tumors. The breakpoint regions are located over a wide area on both chromosomes. To detect these, we developed a PCR method to generate long products. After extraction of genomic DNA (QiaAmp System, Qiagen, Hilden, Germany) DNA was amplified using a mixture of Taq and Pwo polymerases (Boehringer Mannheim, Germany). Several primer pairs from the 5' end of c-myc were tested in each patient.

Lymphoma cells from 20 children with Burkitt's lymphoma and B-ALL characterized by FAB-L3 morphology were examined so far. The product length and the breakpoint regions determined by PCR are listed on the table below.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Primer set location</th>
<th>Breakpoint size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Igh/1q14</td>
<td>1100-1600</td>
</tr>
<tr>
<td>2</td>
<td>Igh/1q32</td>
<td>1400-1900</td>
</tr>
<tr>
<td>3</td>
<td>Igh/1q32</td>
<td>2700-3200</td>
</tr>
<tr>
<td>1</td>
<td>Igh/1q14</td>
<td>3200</td>
</tr>
</tbody>
</table>

*JH: junction region; Sp: switch region; C-d: constant region; CH1: heavy chain constant region

In 11/12 patients with Burkitt's lymphoma and 2/3 with B-ALL the c-myc gene was found in the breakpoint region. PCR products from 1000 to 3700 bp in length were obtained reproducibly. After amplification, the products were characterized by restriction enzyme digestion, hybridization, and in part by direct sequencing.

This PCR based method allows us 1) to detect the localization of chromosomal breakpoints in primary tumor material, 2) to investigate whether distinct breakpoints are associated with treatment outcome and 3) to detect the presence of minimal residual tumor cells during or after therapy.

Supported by Deutsche L¢nkftersforschungsstiftung und Gro¢tdtungsklasse "Molekulare Pathophysiologie des Zellmetastasen"

Utilization of genetic lesions in the treatment of small noncleaved cell lymphomas

Khosh Bhatia, Marin Gutter, Jean-Gabriel Jullde, Hermachenda Venkataseh, Melissa Addie, Gordon Spangler, David Venzon, Elaine Jaffe and Ian Macgash, Lymphoma Biology Section, Pediatric Branch, National Cancer Institute, Bethesda MD, USA

A major objective of basic research in neoplasia is to provide information that will permit the design of novel agents and more appropriate therapeutic modalities. We have utilized molecular lesions that occur in Burkitt's lymphoma to a better define risk categories, to permit dose dependent pharmacological stratification, and to devise tumor targeted therapies, which have been experimentally confirmed. We have demonstrated that variable oligonucleotides directed to these unselected sequences severly restrict the proliferation of BL cell lines without affecting the growth of normal B cells. Additionally, we have utilized the frequent presence of EBV in BL to design two EBV dependent approaches. In the first approach - an EBV dependent enzyme pro-drug system (EDEPS) - we have utilized EBNA-1, a viral transactivating protein invariably expressed in EBV containing cells, to direct the expression of a conditionally toxic gene, cytoxin deaminase (CD). This vector system exploits the presence of latent EBV in tumor cells to induce their selective killing by CD conversion of the pro-drug 5-fluorouracil to the toxic agent 5-fluorouracil. In the second approach, LaSyED (EBV driven lytic system), we have utilized EBNA-1 to direct the expression of an EBV gene (ctb) responsible for the induction of the EBV lytic cycle. This thus provides two levels of specificity for EBV containing cells. Transfection of this expression vector results in cell lysis of the EBV containing but not other cells. Moreover, cell lysis can be achieved without active virus production. Both EDEPS and LaSyED can thus be utilized to allow tumor specific treatment of a spectrum of human malignancies that are associated with EBV.

Use of an anti-ALK antibody in the characterization of anaplastic large cell lymphoma of childhood.

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Anaplastic Lymphoma Kinase (ALK) is a tyrosine kinase inappropriately expressed in lymphoid tissue involved by CD30+ anaplastic large cell lymphoma (ALCL) with the translocation t(2;5)(p23;q35), which juxtaposes the nucleophosphin gene (NPM) with that encoding ALK, resulting in a hybrid (NPM-ALK) message. A polyclonal antibody against residues of the kinase portion of NPM-ALK (designated anti-ALK #1) was tested for clinical utility in paraffin sections of pediatric large cell lymphoma (LCL). Conditions were optimized using fixed and embedded SUP-M2 cells and clinical materials of ALCL. Positive reactions in controls were confirmed by in-situ hybridization using an anisotene ALK riboprobe to detect NPM-ALK transcripts. Antibody analysis of normal autolytic tissue showed strong reactivity only in peripheral nerve, with additional minimal focal binding to connective tissue, particularly in the small bowel and in cartilage of the lung. In routinely processed pediatric lymphomas, 19/20 CD30+ LCL cases (the majority exhibiting anaplastic morphology) labeled with anti-ALK #1, and 5/13 CD30- (3 T-cell, 1 null cell, and 1 B-cell) cases were also ALK+. Sixteen of 17 B-cell pediatric LCL were negative, as were 6 cases of Hodgkin's disease and 7 cases of B-cell lymphoma in adults. There was no significant association between ALK expression and event free survival at two years, similar to the finding reported previously for CD30 expression in these cases. We conclude that the majority of pediatric CD30+ ALCL show ALK overexpression, consistent with the presence of the (2;5)-encoded NPM-ALK fusion, but that the clinical significance of this entity remains unproven.

The role of Epstein-Barr virus in Hodgkin's disease in children from different geographical areas.

Weinreb M, Day PJR, Green PK, Powell JE, Raafat F, Niggl F* and Mann JR, Children's Hospital, Birmingham UK *Department of Pathology, University of Rochester, Rochester, NY, Switzerland

Recent studies have suggested that Epstein-Barr virus (EBV) may play a role in the etiopathology of Hodgkin's disease (HD). To determine the role of EBV in childhood HD in different geographical areas, we used immunohistochemical staining and in situ hybridisation to analyse latent membrane protein 1 (LMP1) and small nuclear non transcribed RNAs (EBER-1) respectively. EBV was tested for in childhood HD from 10 different countries. The proportion of LMP1 positive cases varied significantly being 50% of cases from the UK (38/75), South Africa (9/18), Egypt (7/14) and Jordan (8/16), 60% from the United Arab Emirates (6/10), 70% from Australia (11/16), 81% from Costa Rica (34/42), 88% from Iran (7/8), 90% from Greece (20/22), and 100% of the 56 cases from Kenya. Sensitivity and specific in vitro gene amplification protocols were developed for examining the EBV strain type in archival tissues using several combinations of primers derived from the EBNA2 and EBNA3 coding regions, confirmed by specific restriction endonuclease digestion of the amplified products. EBV strain type 1 was predominant in childhood HD from the UK, South Africa, Australia and Greece. Type 2 was predominant in Egypt. Both EBV strain types 1 and 2 were detected in some cases of childhood HD, Cesta Rica and Kenya. The high incidence of EBV and the presence especially in socio-economic conditions perhaps leading to immunocompromise due to malnutrition. Dual infection is contradictory to the supposed monoclonal EBV origin of HD, however a recent report has detected polyclonal origin of malignant cells within HD tissues.
3. Biology I

CLONAL HETEROGENEITY IN HODGKIN’S DISEASE: RARE OCCURRENCE OF NPMALK FUSION TRANSCRIPTS IN SINGLE HODGKINNREED-STERBERG CELLS BY SINGLE CELL RT-PCR

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The translation (2:q23;q35) fuses the nucleophosmin (NPM) gene to the recently described receptor kinase gene AKL. This leads to the unrestricted expression of the AKL protein in affected tumor cells. The translation is characteristic of a subgroup of CD 30+ large cell anaplastic lymphomas (LACL). Since Hodgkin’s disease (HD) and LACL share many common features, a common pathogenesis has been proposed in a report showing that 11/13 HD carry the NPMALK fusion cDNA (Orscheschk et al., Lancet 1995). This finding could not be reproduced by several other groups. In order to determine if differences in the PCR approaches chosen account for this discrepancy, a single cell RT-PCR assay was developed for the detection of NPMALK transcripts. Single H&RS cells from 9 cases of HD as well as total DNA extracted from single cell suspensions were analyzed by RT-PCR. Specificity of cell selection was shown by the HD-specific pattern of NPMALK expression (EBNA-1 pos., EBNA-2 neg.) in single H&RS cells. In 4/9 cases, NPMALK fusion cDNAs were detected in whole lymph node RNA. In 2/9 cases, NPMALK fusion sequences were amplified from single cells in a very low frequency (1/27 resp. 2/54 cells). These data indicate that the NPMALK fusion protein is not an early step in the pathogenesis of HD. It has to be shown by examining cases in advanced stages or cases that subgroup "transform" if the rare occurrence of NPMALK transcripts in HD represents clonal heterogeneity or if clonal evolution with development of an NPMALK carrying subclone occurs.

REPORT ON THE CONSENSUS MEETING ON LP-HD (COLOGNE, SEPTEMBER 1995)

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Objectives: Lymphocyte predominant Hodgkin’s disease (LPHD) may comprise more than one entity, but diagnostic criteria, clinical features, and treatment strategies are still being controversially discussed. Thus, in December 1994 a multinational project on LPHD was initiated by the European Task Force on Hodgkin’s Lymphoma. This project aimed at the clinical and histopathological characterization of LPHD, as well as its relation to NHL and classical types of HD.

Methods: Paraffin blocks of 525 cases and clinical data of 478 patients from 16 centres were processed in Berlin and Cologne. Before the workshop on LPHD at the Third International Symposium on Hodgkin’s Lymphoma, 232 of these cases could be reviewed by an expert panel without any prior knowledge of clinical presentation or course. Project cases were compared to nodular sclerosis (NS) and mixed cellularity (MC) cases from the German Hodgkin’s Lymphoma Study Group (GHSG).

Results: For 201 cases, both histopathological and clinical data were available. 54.7% were reclassified into nodular paragranuloma (NP), 28.4% into lymphocyte rich classical HD (LRCHD). In general, cases showed the expected marker profile, but in both groups a considerable number of cases with atypical features was observed. Unexpectedly few cases of B cell rich T cell lymphoma could be definitely identified among the cohort. NP differed from NS or MC in terms of histopathology as well as clinical features. NP patients were younger (37 vs. 30 years), more often male (74 vs. 49%), more frequently had stage I or II (74 vs. 57%), but less often mediastinal involvement (10 vs. 80%). NP tumour cells usually stained positive for CD30, but rarely for CD21 and CD15 whereas LRCRD showed the reverse marker profile. Clinical presentation of LRCRD did not differ significantly from NP. Under common treatment for HD, the whole group of unrevised LPHD did not have a better prognosis than the revised GHSG NS HRS, at least at early stage performance better than early stage NS. Though patients with NP frequently released they had a good survival of 95% at 10 years. LRCRD patients had the same prognosis as revised MC cases of the GHSG.

Conclusions: Review of LPHD cases confirmed the generally good outcome of NP patients and gave some clues to explain why the prognosis for unrevised LPHD cases is not better than classical HD. The striking discrepancy between good survival and only average tumour control observed in NP however needs to be further elucidated to understand the underlying biology. Further analysis of all cases submitted must include both histopathological and clinical parameters may be used to identify cases with a better or worse prognosis than average thereby allowing a risk adapted therapy for these patients.

HUMAN EBV NSOPHINS (E0X) EXPRESS FUNCTIONAL CD30 LIGAND AND STIMULATE PROLIFERATION OF HODGKIN’S DISEASE CELLS.


The presence of prominent eosinophilia in Hodgkin’s disease (HD) tissue was first described one century ago and it is now well established that HDs infiltrated with eosinophils degranulation products can be detected in up to 89% of nodular sclerosis and mixed cellularity HD cases. Eosinophilia is partly due to synthesis of cytokines (IL-5, GM-CSF) by Hodgkin’s (H) and Reed-Sternberg (RS) cells. Despite the extensive infiltration in HD tissue, the possible role of E0X in the regulation of H-SF cells growth has not been addressed so far. On the other hand, several evidences suggest that tumor growth in HD may depend on a network of cytokine- and cell contact-mediated interactions among H-RS cells and surrounding reactive cells. Among the surface molecules mediating such interactions, CD30 has been recently characterized as a transmembrane growth factor receptor able to transduce proliferation signals in H-RS cells by engagement with its specific ligand (CD30L). To clarify the biologic significance of eosinophils in HD, E0X from healthy donors and HDs patients with or without eosinophilia (Pit) were isolated to purity (95-98%) and studied for CD30 expression by staining with a monoclonal antibody (4G6) generated against recombinant human CD30 (rhCD30) fusion protein, and rhCD30L. Our results indicate that purified E0X from patients and normal donors showed a strong constitutive expression of surface CD30L and display mRNA encoding for CD30L. Interestingly, E0X from peripheral blood and pleural effusions of patients with active HD and Pit had a higher constitutive expression of CD30L as compared to healthy subjects. Accordingly, CD30L expression was consistently detected by immunohistochemistry on tissue E0X from reactive and HD–involved lymph nodes. In addition, cytokines regulating E0X proliferation and activation (IL-6, IL-3, GM-CSF) were able to enhance the cellular density of CD30L (215,000 cpm) stimulated E0X as compared to L3 (217,500) and 4 (194,500) %, even though the maximal increase of CD30L specific fluorescence (350 % of control) was obtained by a combination of GM-CSF and IL-3. To examine whether naive CD30, on human eosinophils was functionally active in promoting the growth of cultured H-RS cells, titration of phorbol myristate ester (PMA) and TPA induced CD30L expression was able to be completely blocked by soluble CD30-Fc fusion protein.

Our results suggest for the first time that eosinophil in HD may not simply represent “innocent” bystanders but rather participate to the cellular network promoting H-RS cells growth through a CD30L/CDC20-dependent mechanism.

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How Epstein-Barr Virus Controls Cell Proliferation.

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Recombinant EBV based molecular genetic analyses indicate that at least four EBV nuclear proteins, EBNA1, EBNA2, EBNA3A, and EBNA3C and an integral membrane protein, LMP1, are important for efficient primary B lymphocyte growth transformation, while most of the rest of the viral genome can be deleted. EBNA1 is also assumed to be important because of its role in epimorphic maintenance and transcriptional activation. Genetic and biochemical analyses indicate that EBNA2 is a transactivator of transcription that interacts with cellular DNA sequence specific proteins, RBPK and PU.1. EBNA3A and 3C also interact with RBPK. Most of the EBNA2 and much of the EBNA3s in transformed B lymphocytes are associated RBPK. Thus, three EBNA's, EBNA2 and two of the transforming virus are involved in transcriptional regulation in T lymphoma leukemia. This suggests that cell gene products for normal growth are properly regulated through RBPK. The EBV protein, LMP1, is expressed in all EBV transformed cells and in EBV associated malignancies including lymphoproliferative disease, Hodgkin’s disease, and nasopharyngeal carcinoma. LMP1 has transforming effects in many cell types. It contains at least two C-terminal cytoplasmic first 44 and two acids of LMP1 (aa 1-231) are sufficient for primary B lymphocyte growth transformation in the context of the rest of the viral genome. The membrane domains enable LMP1 to aggregate and to mimic a constitutively activated receptor. The C-terminal cytoplasmic tail of LMP1 makes LMP1 similar to the tumor necrosis factor receptor (TNFR) family. Like CD40, LMP1 (1-231) activates NFkB through association with TNF receptor associated factors (TRAFs). LMP1 activates NFkB through TRAF 1 and 2. LMP1 also associates with TRAF3 and that appears to mediate some of the activating effects.

3. Biology I
AN INCREASED INCIDENCE OF LYMPHOMA IN TRANSGENIC MICE OVEREXPRESSING AN EBV-ENCODED HOMOLOGUE OF BCL-2
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The Epstein-Barr virus (EBV) - encoded BHRF1 gene has significant amino acid sequence similarity with the proto-oncogene bcl-2, a gene implicated in the development of human B cell follicular lymphoma. Bcl-2 is a regulator of apoptosis that has been proposed to function via its association with the mitochondrial membrane.

To investigate the functional similarity between BHRF1 and bcl-2 *in vivo* we have established a colony of transgenic mice that constitutively overexpress BHRF1 from an immunoglobulin heavy chain promoter element. These mice develop without any obvious morphological defects. However, histological examination has demonstrated that they have a similar phenotype to bcl-2 transgenic mice. One prominent feature in these animals is the presence of lymphoid hyperplasia, presumably because the BHRF1 gene blocks the normal process of apoptosis in these tissues. With increasing age, bcl-2 transgenic mice develop an excess of high grade lymphomas compared to their control littermates. We have monitored 83 BHRF1 transgenic mice and 41 BHRF1-negative control littermates to assess whether BHRF1 expression has a similar effect. The BHRF1 transgenic mice had a significantly increased incidence of lymphomas compared to their control littermates.

In conclusion we have demonstrated *in vivo* that BHRF1 can function in a manner similar to bcl-2. Overexpression of bcl-2 in follicular lymphoma is thought to be an early event in the pathogenesis of the disease. Our observations suggest that BHRF1 could play a similar role in EBV-associated malignancies such as Burkitt’s lymphoma, Hodgkin’s disease and EBV-positive lymphomas that occur in immunosuppressed individuals.

SELECTIVE LOSS OF INTEGRATED EPSTEIN-BARR VIRUS IN LYMPHOMA HYBRID CELLS POINTS TO IET AND RUN MECHANISM IN EBV ASSOCIATED TRANSFORMATION
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Hodgkin’s disease is seroepidemiologically associated with EBV infection. In contrast only in 50 to 70% of cases EBV can be detected in Hodgkin-Reed Sternberg cells. This observation makes it tempting to speculate that under certain conditions EBV might act in oncogenic transformation by a so called “hit and run” mechanism.

To investigate this hypothesis somatic cell hybrid between the Burkitt’s lymphoma (BL) cell line BL60 and the autologous EBV-immortalized lymphoblastoid cell line IARC 277 were analyzed with regard to physical state of Epstein-Barr virus (EBV) and karyotype changes in long term culture. 6 hybrid clones were long term cultured and compared with hybrid clones early after fusion using conventional cytogenetic, southern blotting and fluorescence in situ hybridization (FISH).

Early after fusion hybrid cells carried EBV genomes of the parental BL cell line integrated near the breakpoint of a translocation chromosome der(19) t(11;19) as well as episomal viral DNA molecules of the parental LCL. During long term cultivation, however, all hybrid cell lines lost the integrated EBV sequence and retained exclusively episomal EBV, whereas in parental BL cells the EBV genomes remained stably integrated. Loss of integrated EBV in all cases resulted from a break proximal to the EBV integration site. Fluorescence in situ hybridization (FISH) revealed that this integration site had become a gap like chromatin area.

We thus conclude, that integration of the EBV genomes constitute a chromosomal region prone to break events akin to the phenomenon of fragile sites. Analysis of the underlying mechanism might reveal new insights into the role of EBV in the transformation process in Hodgkin’s disease.
4. Epidemiology

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS IN KAPOSI'S SARCOMA, MALIGNANT LYMPHOMA AND OTHER DISEASES
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Two novel human DNA fragments were discovered in an AIDS-related Kaposi's sarcoma (KS) lesion using representational difference analysis which permits one to analyze the differences between two complex genomes. These sequences belong to a previously unidentified herpesvirus exhibiting extensive sequence and positional homology with Herpesvirus simiae and partial homology with Epstein-Barr virus. Thus, it is a member of the Cytomegalovirus subgroup of herpesviruses, which are characterized by their ability to replicate in lymphoblastoid cells. This newly discovered virus was named descriptively Kaposi's sarcoma-associated herpesvirus (KSHV) and provisionally designated Human herpesvirus-8 (HHV-8). KSHV is detectable by polymerase chain reaction (PCR) in more than 90% of KS lesions from patients with classical-mediterranean, post-transplantation, endemic-African and AIDS-epidemic KS. In situ PCR studies have demonstrated KSHV in spindle cells and endothelial cells of KS lesions. KSHV can be transmitted to cell blood CD19 positive, B cells by back-transplantation which is blocked by ultraviolet irradiation, which inactivates herpesviral DNA replication and transcription. Thus, KSHV appears to be a transmissible B-lymphotropic herpesvirus. KSHV is detectable in circulating B cells in some HIV-infected patients and this finding appears to predict the future development of KS among these individuals. KSHV has also been consistently identified in an uncommon and usual subset of non-Hodgkin's lymphoma (NHL) related to body cavity-based lymphomas. These lymphomas exhibit a unique constellation of clinical and biological features including presentation as a lymphomatous effusion in the absence of a tumor mass, restricted localization to the body cavity of origin, a distinctive immunophenotype, an indolent course, immunophenotypic, clonal immunoglobulin heavy chain rearrangements, and EBV in the absence of c-myc gene rearrangements. KSHV is uniformly absent from all other forms of AIDS and non-AIDS related NHL. KSHV is also present in the vast majority of AIDS-related, and many cases of non-AIDS-related, multicentric Castleman's disease. This is an asymptomatic lymphoid proliferative disorder believed to be a disease of immune dysregulation in patients who often develop KS and NHL. KSHV is absent from most other HIV- and non-HIV-associated lymphoproliferative diseases. In summary, KSHV is highly associated with all forms of HIV- and non-HIV associated KS, body cavity-based NHLs and multicentric Castleman's disease. Further studies should lead to a better understanding of the role of KSHV in the pathogenesis of these disorders and may eventually show that this virus represents the long sought-after etiologic agent of the enigmatic entity referred to as Kaposi's sarcoma.

TRENDS IN THE INCIDENCE OF LYMPHOMA IN EUROPE
Morgan GI, Jack A, Clydes AD, Brincker JB, Coxheber FJ, Carli PM, Vortoel M, Tumino R, Cartwright RC

A report for the BIOMED NHL Project Group

It has been generally accepted that there has been an increase in the incidence of lymphoma from many cancer registries around the world of around 5% per annum. We have investigated this trend in 8 population-based registries in Europe. These are listed in:
- The LYFO Registry in Odense and the National Cancer Registry in Copenhagen, covering the western part of Denmark (a population of approximately 2.7 million).
- Kupin, Finland, covering Eastern Finland (a population of 0.9 million).
- The Dijon Haematological Registry of the Centre d'Or in Burgundy, France (a population of 0.5 million).
- Two registries from Italy have contributed; from Florence (a population of 1.2 million) and from Reggio in Sicily (a population of 0.2 million).
- The Eindhoven-based Registry of the Comprehensive Cancer Care South in the Netherlands (a population of 0.9 million).
- The LRF Registry in Leeds, covering North and West Yorkshire and Humberside in the UK (a population of 3.6 million).

The data from 1972 to the present day has been collected and grouped according to a number of classifications including local diagnostic codes and the ICD system. To simplify and rationalise the data, it has been aggregated into 9 diagnostic groups which largely conform to the ideas exemplified by the REAL classification. These groups are DCLL, FCLL, HD, mantle cell, other Nodal B, extranodal B, nodal T, extranodal T and lymphoblastic disease. CLL and myeloma have been specifically excluded.

Steady increases in reported incidence of NHL in some registries have stabilised since the mid 1980s. In contrast the incidence of HD is either stable or decreasing. The increase in NHL could reflect either a true increase in incidence or be due to changes in diagnostic trends. To address this problem we have systematically reclassified the pathology of cases from 1986 and 1992.

TRANSREARRANGEMENTS AND THE RISK OF LYMPHOMA MALIGNANCY
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The aspect of our work relevant to "the molecular basis of lymphoma epidemiology" concerns an assay that we have developed which measures "transrearrangements" between rather than within antigen receptor loci. Every individual has such rare rearrangements within occasional peripheral blood lymphocytes. The level of such rearrangements is probably a reflection of a person's inherent (inherited) genomic instability, plus the acquired "destabilizing" influences that a person encounters.

The level of transrearrangements is markedly increased in patients with the autoimmune reductive disease, Sjogren's disease. Such patients also have a predisposition to the development of lymphoma. The level is also increased in agriculture workers exposed to herbicides, pesticides, and fungicides. This population also shows an increased risk of NHL. The level is also increased in patients with Hodgkin's disease receiving chemotherapy. Thus, it appears that this assay is a kind of biomarker for genetic instability in general, and possibly certain kinds of cancer risk as well. With regard to lymphomagenesis it is not just a fortuitous biomarker, because the mechanism that causes transrearrangements, V(D)J recombination, is, at least in part, the same mechanism that is responsible for the majority of chromosomal translocations associated with the development of lymphoma malignancy. Other assays of genetic instability (glycophorin A, HPRT) show related or confirmatory results.

We are continuing to test this assay in various human study populations. We have also developed a murine model so that we can do more refined genomics and exposure assessments than with human populations. We have already discovered certain mouse mutations which demonstrate an increased level of transrearrangements which can be amplified by certain environmental carcinogens. Preliminarily, in these mutant mice the increase in transrearrangements seems to predict subsequent development of lymphoma malignancy.

Paolo Vincis (*) Adare Seniori Costantini (**) Working for the Epidemiology of Hematolymphopoetic Malignancies in Italy
Italian case-control study on leukemias-lymphomas

The Italian multicenter study on hematolymphopoetic malignancies, funded by the U.S. National Cancer Institute (CAS1086) has been completed and partially analyzed. The areas included in the study are: a) two industrialized areas with a concentration of both mechanical and chemical industries (town of Torino, province of Varese); b) seven agricultural areas, with different types of agricultural activities (provinces of Novara, Vercelli, Alessandria, Imperia, Ragusa, Siena, Forli); c) the province of Firenze, with a considerable activity in the field of leather goods and shoe production, and the province of Verona, with a mixture of industrial and agricultural activities. Overall, the study covered a population of approximately 7 million residents. The design is a population-based case-control study, i.e., it collected all the newly diagnosed cases of leukemia, lymphoma and myeloma in residents in the participating areas (males and females), and a control group represented by a random sample of the general population. The main study hypotheses were 1) the association between herbicide exposure and non-Hodgkin lymphomas, and 2) the association between solvent exposure and Non-Lymphocytic Leukemias (NLI), plus other hypothesis- generation goals. We have interviewed approximately 2700 cases and 1800 controls and we will show preliminary results.
PATHOGENESIS OF GASTRIC LYMPHOMA: THE ENIGMA IN HONG KONG

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Helicobacter pylori has been shown to be closely associated with gastric lymphoma and a causal relationship has been postulated. To date, all supportive evidence has been collected from studies done on the Western populations. We carried out a retrospective study on 53 cases of primary gastric non-Hodgkin’s B-cell lymphomas (B-NHL) from Hong Kong Chinese patients using Warthin-Starry silver staining for evidence of H pylori infection. H pylori colonization was found in 31/53 (62%) whereas the association of duodenal associated lymphoid tissue (MALT), 14/27 (52%) high-grade MALT lymphoma, 7/13 (54%) high-grade B-cell lymphoma without features of MALT lymphoma: The overall positivity rate in gastric lymphomas was 29/53 (55%), and 22/40 (55%) in MALT lymphoma as a group. None of the three groups of gastric lymphoma nor gastric lymphoma as a whole show a significantly higher infection rate as compared with that found in healthy Chinese blood donors as detected by serological assay (55%), or compared with that in endemic biopsies from non-ulcer dyspeptic patients (52-57%). These results are in contrast to the high positivity rates of H pylori reported in primary gastric B-NHL lymphoma in the West (92-100%). As Epstein-Barr virus (EBV) has also been reported in gastric lymphoma, we also screened for EBV in these 53 cases using in situ hybridization with oligonucleotide probes for the EBV encoded small RNA (EBER1 and EBER2). EBER2 was found in tumor cells in only one case which was H pylori negative. Based on our data and a careful review of the literature, we are of the view that very few gastric lymphomas are associated with EBV. Besides H pylori and EBV, it has been suggested that occupational exposure to pesticides and solvents may play an important role in the pathogenesis of gastric lymphoma in Italy (Pagliolo et al., Haematologica 1994; 79: 213-217).

These results from different countries suggest that the etiology and pathogenesis of gastric lymphoma may vary in different populations. In the Hong Kong Chinese population, although gastric lymphoma is common, further investigations are required to elucidate the possible pathogenic factors.

Normal and Neoplastic B Cell Development
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It is no surprise that the development of antibody forming cells (B lymphocytes) is primarily guided by genetic mechanisms which allow the expression of antibodies of a single and appropriate specificity in a given B cell. B cell generation from stem cells in the bone marrow is achieved through a programme of gene rearrangements in the immunoglobulin (Ig) heavy and light chain loci. In this process, the cells have to pass "checkpoints" where they are controlled for successful performance in this developmental pathway. Cells lacking in-frame Ig gene rearrangements or expressing autoreactive antibodies are eliminated. After this initial selection, the cells emigrate into the peripheral immune system and enter a resting state. When B cells are recruited by antigen into antibody responses, they can differentiate along several lines. One pathway is the generation of memory cells in germinal centers. There the cells again enter a phase in which their Ig genes are modified by genetic mechanisms, this time by somatic hypermutation. During extensive proliferation large numbers of somatic antibody mutants are generated, and only cells expressing antibodies with a high affinity for the antigen survive. As in the bone marrow, cells expressing inappropriate antibodies are efficiently deleted. The high affinity memory B cells emerging from the germinal center response are again resting cells, waiting for renewed encounter of antigen. Malignant transformation can occur at any developmental stage, most prominently, however, in the phases where the genetic material of the cells is inherently subject to somatic changes. The origin of the resulting tumor cells can often be traced back to the original progenitor, through molecular analysis of the rearranged antibody genes. In such an analysis, carried out in collaboration with the groups of M.-L. Hansmann and V. Diehl, Hodgkin-Reed-Sternberg (HRS) cells in Hodgkin's disease isolated by micromanipulation from tissue sections, were found to belong to the B cell lineage in at least the majority of cases and in general to derive from a single progenitor cell whose state of differentiation will be discussed.
5. Review lectures

DECISION-MAKING FOR THERAPEUTIC OPTIONS IN FOLLICULAR LYMPHOMA
T.A. Lister - ICRF Dept. of Medical Oncology, St. Bartholomew's Hospital, London, England

There are several opportunities for therapeutic decisions in the management of the patient with follicular lymphoma. What are the indications for instituting therapy in the first place? How aggressive should the approach be? Should clinical remissions be consolidated?

Against the background of a repeatedly cytotoxic therapy responsive disease, the major questions are 'is it realistic to treat with curative intent?' 'is biological therapy relevant?'

These issues will be discussed.

CURRENT SITUATION IN THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

CLL is the most frequent form of leukemia in Western countries. The majority of patients with CLL are more than 65 years-old at diagnosis. The course of the disease is highly variable and, at present, there is no curative therapy for CLL. All these factors should be carefully considered when deciding therapy in patients with CLL. Staging systems and prognostic factors allow to make treatment decisions on the basis of the individual risk of each subject. Patients with low-risk disease may survive as long as normal individuals and should not be treated unless the disease progresses. By contrast, patients with poor risk factors (i.e., advanced clinical stage, diffuse bone marrow infiltration, high- and rapidly increasing WBC counts) have a median survival of less than 5 years and require treatment. CLL therapy has evolved around the use of alkylating agents, with no substantial improvement in therapy over the last 20 years. A number of situations (e.g., autoimmune hemolytic anemia, hypersplenism) require specific treatment approaches (e.g., corticosteroids, splenectomy). While symptoms palliation and prolongation of survival still are reasonable goals of therapy in most patients, new treatment modalities (i.e., purine analogues, biotechnology, transplants) offer great promise. These latter approaches are likely to improve the outcome of patients with CLL and might even cure some of them.

Can we improve upon the International Index? Margaret A. Shipp, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

The International Index is currently used to identify appropriate patients for risk-related treatment strategies. However, the International Index is based on clinical features that are surrogate variables for the biological heterogeneity of aggressive NHLs. Increased understanding of the biological heterogeneity of aggressive NHLs is likely to lead to more refined prognostic models and to biologically-based approaches to therapy. In recent years, cellular and molecular features including tumor cell proliferation, immunophenotype, adhesion molecule expression, and karyotypic abnormalities have been linked to survival in aggressive NHLs. Certain biologic features have independent prognostic significance and others have been associated with previously described clinical features such as performance status, stage of disease, and extranodal sites of disease have now been associated with biologic variables including circulating IL-6 levels, tumor cell surface CD44 expression, and bcl-6 rearrangements, respectively. Additional biologic variables with independent prognostic significance include indices of tumor cell proliferation such as Ki-67 expression, bcl-6 rearrangements, and the expression of variant isoforms of the CD44 cell surface adhesion molecule. Additional biologic variables have also been associated with adverse outcome, including overexpression of the bcl-2 protein (independent of bcl-2 chromosome rearrangements) and the presence of a T cell surface phenotype. In addition, host immunocompetence is likely to play an important role in the response to therapy of aggressive NHL. Taken together, the emerging data prompt speculation that we will eventually be able to replace the clinical prognostic factors in our current models with more relevant cellular and biological features and to use these features to develop novel approaches to the disease.

Long Term Toxicity of Treatment for Hodgkin's Disease
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The majority of patients treated for Hodgkin's disease (HD) are cured. 2475 consecutive patients seen and treated at Stanford University between 1962 and 1995 had an actuarial risk of death from HD of only 19.5% at 30 years. However, the actuarial risk of death from other causes was nearly 50%. Although the overall actuarial survival was 40% at 30 years and the actuarial survival with deaths from HD censored was 50%, the expected survival for an age- and sex-matched population is 85%. The major causes contributing to excess deaths in our patient population were second cancers and cardiovascular disease. Second cancers included leukemia (relative risk 6.4; absolute risk 18.4%); non-Hodgkin's lymphoma (RR 35.3; AR 16); and solid tumors (RR 4.3; AR 46.8). Secondary leukemia was primarily ANLL (37/42); there were also cases of myelodysplastic syndrome. These were related primarily to exposure to MOPP (or MOPP-like) chemotherapy. Secondary NHL was primarily diffuse large cell (24/39) or other B-cell (13/39). This was not related to any specific aspect of therapy. Solid tumor risk was significantly elevated for lung/pulmonary, breast, melanoma, soft tissue, bone, stomach, salivary gland, and thyroid. Lung cancers occurred primarily in smokers. The risk for breast cancer decreased with increasing age and was greatest in women who were younger than 20 at the time of treatment. Solid tumors were most common in patients whose treatment included irradiation. Cardiac mortality (RR 2.9) often was due to coronary artery disease (55/68) and occurred primarily in patients who had received mediastinal irradiation. Non-coronary artery causes of cardiac mortality have been reduced during the 30 years of patient treatment as a result of changes in irradiation techniques. These observations emphasize the importance of long-term follow-up after treatment for HD.
CHOP IS THE CURRENT STANDARD FOR THE TREATMENT OF HIGH RISK PATIENTS WITH DIFFUSE LARGE CELL LYMPHOMA

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Therapy for aggressive non-Hodgkin's lymphomas has undergone significant evolution in the last 25 years. First generation combination chemotherapy studies produced complete response rates (CRs) of 45-53% with 30-37% long term survivors. New treatment programs aimed at increasing CRs were then developed with the assumption that the additional CRs would also become long term disease free survivors. Initial reports of single institution pilot studies with third generation regimens suggested 68-86% CRs and 58-69% survival; however, with longer follow-up, the survival decreased. Furthermore confirmatory national Phase II trials using these newer regimens produced CRs of only 49-65% and survival of 50-61%. Thus ultimate conclusions concerning the efficacy of these new regimens awaited the results of prospective randomized trials. The Southwest Oncology Group (SWOG) conducted a randomized trial comparing standard therapy, CHOP, to the third generation chemotherapy regimens, mBACOD, ProMACE-CytaBOM, or MACOP-B. After six years, there is still no difference in response rate, time to treatment failure, or overall survival between CHOP and the third generation regimens. However the cost and toxicity of the new regimens were higher. Based on the fact that fewer than 50% of these patients are cured, the best approach for any patient is an experimental approach designed to improve our ability to cure the disease. Examples of this include: 1) increasing the dose intensity of drugs used in standard regimens, 2) preventing the development of drug resistance, and 3) autologous bone marrow transplants and or peripheral stem cell support as rescue from marrow ablative chemotherapy. If a patient is not eligible or doesn't wish to participate in a clinical trial, CHOP as inadequate as it is, remains the gold standard.

IS HIGH-DOSE BETTER THAN STANDARD-DOSE CHEMOTHERAPY AS INITIAL TREATMENT OF POOR-RISK LARGE-CELL LYMPHOMAS? A CRITICAL ANALYSIS FROM AVAILABLE RANDOMIZED TRIALS


Following the initial trial by Philip and colli. (N Engl J Med 316, 1493, 1987), several studies have shown that high-dose treatments requiring bone marrow support can cure patients with relapsed/refractory lymphomas otherwise incurable with standard-dose regimens. However, until recently high-dose regimens have been employed almost exclusively as salvage treatments, mainly as a consequence of their marked morbidity, mortality, requirement for specialized patient care, and costs. This condition dramatically changed in the late 80's, following improvements in patient care, and in particular the introduction into clinical use of the hematopoietic progenitor cells elicited to circulate in the peripheral blood by growth factors like G-CSF or GM-CSF. A few randomized studied have since been carried out, reporting either superior results for the high-dose arm, or no significant difference. These conflicting findings might reflect, at least in part, the selection criteria adopted leading to enrollment of highly selected patient subsets, as well as, possibly, to the therapeutic strategy universally adopted (i.e. use of one high-dose consolidation course followed multiple standard-dose cycles). In 1987, in collaboration with the University of Turin, we started a randomized cross-over study to compare MACOP-B with high-dose chemotherapy given as initial treatment to patients with high-risk diffuse B-cell lymphomas. Fifty-one patients were entered into the MACOP-B, and 48 into the high-dose arm. The 7-year results clearly indicate that high-dose is more effective than standard-dose therapy, and that the strategy of giving high-dose upfront is superior to its use as salvage treatment, yielding a significantly higher overall survival rate. A definitive assessment of the superior activity of high-dose chemotherapy in poor-risk lymphoma patients, and of its value in a more general setting, would require validation in the context of larger multicenter, randomized trials.
PATHOGENETIC AND PROGNOSTIC ASPECTS OF CUTANEOUS LYMPHOMAS

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The development of a cutaneous T-cell lymphoma (CTCL) is a relatively rare event presenting peculiar features. These include the preferential homing of lymphocytes in keratinizing epidermal structures of the skin. The slow progression of the disease through various stages may be explained by a stepwise accumulation of DNA repair gene-, oncogene-, or tumor suppressor gene-mutations.

The difficulties in culturing the neoplastic cells in vitro may be due to the special microenvironmental requirements needed, which include keratinocytes and dendritic cells and the cytokines produced by these cells.

Many of the clinical phenomena seen in CTCL, such as increased risk of second malignancy, elevated IgE and IgA serum levels, or hypereosinophilia may be explained by a switch from T helper 1 cells to T helper 2 cells during tumor development.

The most important prognostic parameters in CTCL after TNM-stage (skin tumor burden) are the histologic grade of malignancy, age at diagnosis, and the duration of the prediagnostic phase. They have to be considered when therapeutic approaches are planned.

CLINICAL AND PATHOLOGIC ASPECTS OF CUTANEOUS B-CELL LYMPHOMAS

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Primary cutaneous B-cell lymphomas are not as rare as is generally believed. They are defined as malignant B-cell proliferations presenting with cutaneous involvement alone and no evidence of extracutaneous manifestations over a period of at least 6 months when complete staging has been performed.

The major subtypes are follicle center cell lymphoma of the head and trunk, immunocytoma and large B-cell lymphoma of the leg (EORTC-Classification 1996). These lymphomas are distinct entities which are different from nodal lymphomas in clinical and functional behaviour. Of special interest is the recently recognized marginal zone B-cell lymphoma.

New techniques - immunophenotyping, molecular genetic analysis, laser-based single cell microdissection - have largely improved our understanding of the morphology and pathogenesis of this important group of diseases.

Lymphomatoid papulosis and anaplastic large cell lymphoma of the skin.

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Lymphomatoid papulosis (LyP) and primary cutaneous Ki-1+ anaplastic large cell lymphoma (ALCL) comprise a spectrum of related diseases in which spontaneous regression is observed. Principal distinguishing features between LyP and ALCL relate to lesion size (<2cm in LyP), ratio of Ki-1+ atypical cells to inflammatory cells (lowest in LyP) and extent of infiltrate (greatest and extending into the subcutis in ALCL). Atypical cells are activated helper T cells which have an aberrant phenotype, CD7-. Cytogenetics reveals multiple chromosome abnormalities with recurrent breakpoints at 1p36 and 10q24-26; (6)(2;5)(p23;q35) is absent or infrequent. Individual skin lesions contain a dominant T cell clone and separate lesions are clonally related. Multiple cytokines, e.g. TNF-α, TGF-β, are produced by the Ki-1+ clone and may influence the histology and regression of skin lesions. Inactivation of the TGF-β surface receptor complex may be a mechanism of tumor progression. Low dose methotrexate (10-25mg weekly) is effective therapy for LyP and selected ALCL. Expression of CD30 is associated with a better prognosis in primary cutaneous lymphomas, regardless of cytologic type. Extracutaneous disease, which occurs in 25% of ALCL, is associated with poor survival. The etiology of LyP and Ki-1+ ALCL are unknown; EBV does not appear to be involved in LyP.
CLASSIFICATION OF CUTANEOUS LYMPHOMAS: REAL VS. UN-REAL

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The REAL classification proposed by the ILSG does not deal separately with cutaneous lymphomas. However, the basic premise of the classification is the recognition of disease entities, through the integration of histopathology, clinical features, immunophenotype, and molecular findings. Therefore, if cutaneous involvement is a unique aspect of a disease entity, the clinical fact is considered integral to disease recognition.

This author recognizes several types of lymphoma, for which cutaneous involvement is essential, i.e., the disease is not recognized in a non-cutaneous form. These include 1) mycosis fungoides/Sézary syndrome and its variants; 2) lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma (ALCL); and 3) subcutaneous panniculitis-like T-cell lymphoma. Other forms of T-cell malignancy may frequently involve the skin, such as classical ALCCL, adult T-cell leukemia/lymphoma, T-cell lymphoma, and angiocentric and non-angiocentric T/NK cell lymphomas and leukemias.

Among B-cell malignancies there is no disease entity for which cutaneous involvement is clearly essential, although one possible example is cutaneous follicle center lymphoma (CFCCL). CFCCL differs from nodal FCL both phenotypically and genotypically (bcl-2-negative). This disease is most common in the skin, but is seen rarely in other extranodal sites. "Cutaneous immunocytoma" is a variant of MAL-T-type or marginal zone B-cell lymphoma. It mirrors well the features of MAL-T-type lymphomas seen in other extranodal sites. The terminology of cutaneous MAL-T-type lymphoma is preferred, because immunocytoma implies a systemic disease, with different clinical implications. Other B-cell lymphomas with frequent cutaneous involvement include: B-cell precursor lymphoblastic lymphoma/leukemia, intravascular or angiotropic lymphomas, as well as other large B-cell lymphomas.

The principles of the REAL classification are applicable to cutaneous lymphomas, as well as to lymphoid malignancies in other anatomic sites. Organ-specific classification schemes are not required, and may impede the recognition of common features of diseases involving multiple anatomic sites.

DIAGNOSTIC AND THERAPEUTIC CONCEPTS IN CUTANEOUS LYMPHOMA: A DERMATOLOGIST'S PERSPECTIVE

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Certain diagnosticists argue that clinically benign conditions such as purpurae or plaques (PEP) should be included with mycosis fungoides (MF) in the cutaneous T-cell lymphoma spectrum. However, the therapeutic and prognostic ramifications of this viewpoint need to be carefully considered. With the recent availability of sensitive molecular techniques to detect T-cell clones in cutaneous infiltrates, it may be possible to differentiate between these entities. However, it is my perspective that PEP and MF cannot be reliably differentiated using available clinical and pathologic criteria, and that these entities are therefore better viewed as part of a continuum of cutaneous lymphoproliferative disease with varying potential for tumor progression and terminal transformation into high grade lymphoma. This concept considers both PEP and patch phase MF as initial non-aggressive stages of an abnormal lymphoproliferative process that may progress into plaque to tumor phase MF depending on as yet poorly understood genetic changes in the tumor cells or loss of anti-tumor effector mechanisms. Skin-directed chemotherapy or radiotherapy seem to be curative in 30-50% of patients with limited extent of skin involvement, but may also be effective in patients with more advanced disease by reducing the number of abnormal lymphocytes in the skin that might in time acquire a more malignant growth potential. Biological response modifiers and cytotoxic agents selectively targeted against T-cells are seen as the most promising therapeutic approach for patients with MF in the immediate future.