B AND T CELL LYMPHOMAS

Organizers: Robert Peter Gale, James Armitage, Thomas Waldmann and Franco Cavalli

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Keynote Address

T 001 THE ROLE OF VIRAL FACTORS AND CHROMOSOME TRANSLOCATIONS IN THE GENESIS OF HUMAN AND RODENT B-CETHLL LYMPHOMAS. George Klein, Department of Tumor Biology, Karolinska Institute, S-104 01 Stockholm, Sweden.

This talk will focus on human, mouse and rat B-cell derived lymphomas and plasmacytomas, respectively, that regularly carry aN Ig/myc translocation.

The discussion on the **viral factors** will deal with the role of EBV in the genesis of Burkitt lymphoma (BL) and other neoplasms. Our recent discovery that one of the EBV-encoded proteins complexes with both Rb and p53 will be presented in detail and contrasted with similar complexing found in the SV40, adeno- and papillomavirus systems. For the mouse plasmacytomas, the accelerating role of Abelson virus will be briefly considered.

The **chromosomal translocations,** that lead to the juxtaposition of Ig and myc sequences will be surveyed and updated with the following new findings:

i. Genetic susceptibility of BALB/c mice to PC-genesis is at least partly determined at the cellular level and affects translocation proneness;

ii. The translocation can occur very early in B-cell development, without interfering with differentiation;

iii. p53 mutation is at least one of the secondary events that may promote the outgrowth of the Ig/myc translocation carrying tumor;

iv. Expression of wild type p53 from a temperature sensitive construct introduced into p53 mutation carrying murine or human tumor cell lines induces apoptosis. We suggest that the suicidal program is elicited by the contradictory signalling of Ig activated myc and growth-arresting wtp53.

Normal B and T Cell Development

T 002 B CELL DEVELOPMENT IN NORMAL AND MUTANT MICE, Fritz Melchers, Hajime Karasuyama, Dirk Haasner, Ulf Grawunder, and Antonius Rolink, Basel Institute for Immunology, Basel, Switzerland.

V_{preB} and λ_5 are two genes which are selectively expressed in pre B-lymphocytes, and which encode two proteins that associate noncovalently with each other to form the surrogate light chain. The surrogate L chain is expressed on the surface of pro and pre B cells together with either p130/p55, with $D_HJ_HC\mu$-protein, or with μH chains at different stages of B cell development. Mice, in which the λ_5 gene has been disrupted by targeted integration of a defective gene (λ_5T), are used to define the defects in B cell development introduced by the lack of expression of the surrogate L chain. The sizes of the early pro and pre B cell pools with H chain genes in germline or D_HJ_H-rearranged conformations, and their rates of differentiation to sIg^+ cells "in vitro" and "in vivo" are normal in bone marrow of λ_5T mice. H chain loci DHJH-rearranged in reading frame II are not suppressed. The largest compartment of pre B cells in bone marrow which appears positively selected for cells with productively rearranged H chain genes is absent in λ_5T mice, indicating that the complex of μH chain and surrogate L chain is involved in filling this pre B compartment from which most of the B cells are generated each day in normal bone marrow.

B and T Cell Lymphomas

T 003 CLASSIFICATION OF T-CELL LYMPHOMAS, Karl Lennert, Zentrum Pathologie und Angewandte Krebsforschung, University of Kiel, Niemannsweg 11, D-2300 Kiel, Germany

There are a few classifications of T-cell lymphomas, which are usually less differentiated than ours. We developed our classification at some conferences held with Japanese, Chinese and European lymphoma experts on the basis of a large number of cases from Asia and Europe. It is described *in extenso* and illustrated in our latest monograph.[1]

We classify the T-cell lymphomas (like the B-cell lymphomas) basically according to the morphologically and immunologically defined cell type. The result is a basic division into T-cell lymphomas of precursor (thymic and prethymic) type and T-cell lymphomas of peripheral type. The precursor cell lymphomas are designated as lymphoblastic lymphomas. Morphologically they cannot be distinguished from T-ALL.

A basic criterion for distinguishing the peripheral T-cell lymphomas is the size of the cells: small cell T-cell lymphomas, which can also contain some large cells, are combined in the group of low-grade T-cell lymphomas. Medium-sized cell and large cell T-cell lymphomas are included in the high-grade category. Accordingly the malignancy grade is defined morphologically and not clinically.

The low-grade lymphomas include two variants that evidently produce lymphokines and therefore reveal an admixture of different cells: lymphoepithelioid lymphoma (LeL) shows clusters of epithelioid cells; the AILD type can display admixtures of eosinophils, basophils, plasma cells and epithelioid cells. A special feature of this lymphoma is the proliferation of large complexes of follicular dendritic cells.

Lymphoblastic lymphoma is listed at the end of the high-grade lymphomas. In addition in the high-grade group large cell anaplastic lymphoma deserves special attention because of its Ki-1 (CD30) positivity and its characteristic chromosome anomaly (t[2;5]). This is the malignant lymphoma that is most responsive to treatment.

T-cell lymphomas differ from B-cell lymphomas in the following manner:

1. They are less common (in Western countries only ≈ 20% of the malignant lymphomas).
2. They show the following morphological characteristics, which are only partially T cell specific, but which, taken together, allow us in many cases to identify T-cell lymphomas as such without having to apply immunohistochemistry or molecular genetics: (a) they show primary infiltration of T regions; (b) epithelioid venules are often increased in number and sometimes atypical; (c) the tumor cells are mostly pleomorphic, rarely basophilic; sometimes "clear" cells are found and occasionally giant cells, including the Sternberg-Reed type; in imprints there are very often admixtures of tumor cells with azurophil granules and (d) sometimes an admixture of interdigitating cells, epithelioid cells, eosinophils and/or plasma cells (polytypic) may be seen.
3. Characteristic of peripheral T-cell lymphomas is a marked tendency to change their morphology and immunophenotype. Different grades of malignancy can also be observed at the same time in various localizations.

Reference

1. Lennert K, Feller AC (1992) Histopathology of non-Hodgkin's lymphomas. Springer, Berlin Heidelberg New York

T 004 **CLASSIFICATION OF B-CELL LYMPHOMAS, Bharat N. Nathwani, M.D., University of Southern California School of Medicine, 2011 Zonal Avenue, HMR 204, Los Angeles, CA 90033.**

By definition, B-cell lymphomas show one or several B-cell markers---surface and/or cytoplasmic immunoglobulin, CD19, CD20, CD21, CD22, CD23, CD24. B-cell lymphomas have origin from one of four compartments of the lymph node---(1) follicular center, (2) mantle zone, (3) marginal zone, and (4) interfollicular area. The patterns of infiltration, the cytologic features of the neoplastic cells, as well as immunophenotypic and genotypic features, of the various lymphomas correlates well with the compartment from which they arise. (1) Follicular center cell lymphomas form follicular and/or diffuse patterns. These lymphomas are usually CD10+, CD5-, Bcl-2+, Bcl-1-, and exhibit the t(14;18) translocation. They are classified in the Kiel classification as (a) centroblastic-centrocytic with (i) few or (ii) many centroblasts, and (b) centroblastic. In the Lukes and Collins classification, they are classified as (a) small cleaved, (b) large cleaved, (c) small noncleaved, and (d) large noncleaved. (2) Mantle cell lymphomas may have mantle zone, mantle cell nodular and/or diffuse patterns. These lymphomas are usually CD10-, CD5+, Bcl-2-, Bcl-1+, and often exhibit the t(11;14) translocation. In all classification systems, they are classified as mantle cell lymphoma. (3) Marginal zone lymphoma arises from cells that surround the mantle zone. This lymphoma may have a marginal zone pattern or other patterns of infiltration, but has yet to be fully characterized and understood. (4) Interfollicular B-cell lymphomas may be classified as follows: a) monocytoid B-cell lymphoma (MBCL). This lymphoma is unique because of its distinctive morphology and a close relationship with follicles. It may surround reactive follicles, invade follicles and replace follicular center cells, or show partial differentiation towards follicular center cells. In addition, in this lymphoma, there is a high incidence of "composite" lymphomas (presence of other subtypes such as lymphocytic, plasmacytic, mantle cell and follicular). These lymphomas are CD10-, CD5-, Bcl-2-, Bcl-1-. MBCL is closely related to the low grade B-cell type of mucosal-associated lymphoid tissue (MALT) lymphoma and marginal zone lymphoma. (b) B-CLL (small lymphocytic lymphoma). This lymphoma usually exhibits pseudofollicular growth centers (pseudofollicular pattern) and is CD10-, CD5+, CD22+, CD23+, Bcl-2-, Bcl-1-. (c) immunocytoma (plasmacytoid lymphocytic lymphoma) which is CD10-, CD5-, CD22+, CD23+, Bcl-2-, Bcl-1-. (d) plasmacytoma which is CD10-, CD5-, CD22-, CD23-, Bcl-2-, Bcl-1-. (e) B-immunoblastic lymphoma which is CD10-, CD5-, CD22+, Bcl-2-, Bcl-1-. (f) Burkitt's lymphoma which is CD10+, CD5-, CD22+, C-myc+, and usually exhibits the t(8;14) translocation. (g) B-lymphoblastic lymphoma, which is Tdt+, CD10+, CD19+, CD20+. (h) anaplastic large cell lymphoma which is CD19+, CD20+, and CD30+.

Etiology

T 005 **THE EPIDEMIOLOGY OF HODGKIN´S DISEASE, Volker Diehl and Andreas Engert, Medizinische Universitätsklinik I, 5000 Köln 41, Germany**

Despite many advances in the characterization of the Hodgkin and Reed-Sternberg (H-RS) cell, the pathogenesis of Hodgkin´s disease (HD) is still an enigma. Epidemiologic research has revealed unique features including a bimodal age distribution with peak incidences in the late twenties and the early sixties. This pattern shows socioeconomically determined variations: In poor countries and rural aereas, HD is generally less common but the incidence prior to the age of 15 years is higher than in developed countries. By contrast, in developed countries and highly educated settings, HD is more frequently affecting young adults thereby relatively spearing children. This pattern resembles the behaviour of infectious diseases thus supporting the theory that HD may develop as a consequence of latent virus infection. The epidemiological features support the concept of the heterogeneity of HD resulting from different etiologies in different age groups. On the other hand, reports on familial clustering and HLA correlations are regarded as evidence of a genetic factor in the pathogenesis of HD. In addition to genetical factors, social conditions affecting the probability of exposure to the infectious agent and the immune status of the host might explain the considerable variability.

Since the observation of a higher risk for the development of HD in patients with a history of infectious mononucleosis, Epstein-Barr virus (EBV) has been discussed as a possible causative agent. With the application of more sensitive molecular biological methods, EBV genome can now be demonstrated in H-RS cells in up to 70% of patients with HD. EBV is known for its capacity to immortalize B-lymphocytes. The transformation of EBV infected cells into malignant H-RS cells has been reported in a patient repeatedly treated with steroids. Interestingly, Hodgkin-like lesions were observed after the transplantation of material containing EBV-negative H-RS cells and EBV-positive bystander-cells in SCID mice.

Other features that have been discussed as possible risk factors for HD include exposure to carcinogenic substances (dioxin, cigarette smoke), presence of certain HLA locus alleles (HLA B18, HLA DR5), other viruses (HHV-6), and disturbances in the immunologic surveillance. The higher incidence of HD in AIDS patients maybe used as a tool to further investigate mechanisms involved in the pathogenesis of HD.

T 006 **EPIDEMIOLOGY OF NON-HODGKIN'S LYMPHOMA,** Dennis D. Weisenburger, University of Nebraska Medical Center, Omaha, NE 68198.

Between 1973 and 1989, the incidence of non-Hodgkin's lymphoma (NHL) increased by nearly 60% in the United States, one of the largest increases of any cancer. In contrast, the incidence rates of Hodgkin's disease and leukemia were stable during this period. In 1992, over 40,000 persons were diagnosed with NHL and approximately 20,000 died of the disease. Although much attention has been devoted to NHL arising in persons with acquired immunodeficiency syndrome (AIDS), most of this increase in NHL cannot be attributed to AIDS. However, by the mid-1990's, AIDS will be a major cause of NHL, accounting for 10 to 15% of all new cases. The annual incidence rate of NHL per 100,000 persons in the United States has risen from 5.9 in 1950 to 9.3 in 1975 and to 13.7 in 1989. The increase has occurred in both males and females, and in whites more than blacks, with age groups over and under 65 years both showing increases of over 50% since 1973. The incidence rate in 1989 was 56% higher in white males (17.8) than white females (11.4). The largest increase in incidence has occurred in the elderly, and rates have been increasing more rapidly in rural areas than urban areas. Histologically, the largest increases have occurred in the diffuse large cell and immunoblastic categories, and there has been a disproportionate increase in extranodal lymphomas. Similar findings have also been reported in other developed countries. Epidemiologic studies have suggested that environmental factors may play an important role in the etiology of NHL. Occupational studies have found that persons with certain types of jobs have an increased risk of NHL, including farmers, pesticide applicators, grain millers, meat workers, wood and forestry workers, chemists, painters, mechanics, machinists, printers, and workers in the petroleum, rubber, plastics and synthetics industries. Thus, chemical exposures of various types may be etiologic for NHL. Chemicals that have been linked to the development of NHL include a variety of pesticides (2,4-D, organophosphates, chlorophenols), solvents and organic chemicals (benzene, butadiene, carbon tetrachloride, carbon disulfide), wood preservatives (creosote, pentachlorophenol), drugs (alkylating agents, immunosuppressives, hydantoins), dusts (wood, cotton), and some components in hair dyes. In a recent study in Nebraska, we found that male farmers who frequently used the herbicide 2,4-D had an over 3-fold increased risk of NHL. Similarly, frequent organophosphate insecticide use, adjusted for 2,4-D use, was also associated with a 3-fold increased risk of NHL. For women, the use of hair coloring products, particularly permanent and dark coloring products, was associated with a 1.5- to 2.5-fold increased risk of NHL. These findings indicate that additional epidemiologic studies are needed to elucidate and quantitate etiologic factors for the current epidemic of NHL.

Molecular Biology-I

T 007 PREDICTION AND STUDY OF LYMPHOID MALIGNANCY MEDIATED BY INTERLOCUS SITE-SPECIFIC RECOMBINATION, Ilan R. Kirsch[1], Donald P. Lombardi[1], William N. Fishbein[2], and Trang Huong[3], [1]N.C.I.-Navy Medical Oncology Branch, Bethesda, MD 20889-5105, [2]Armed Forces Institute of Pathology, Washington, D.C.,[3]Clinical Research Institute, Montreal, Canada

We have developed an assay for a type of lymphocyte-specific genetic instability that is based on a quantitation of the frequency of a particular "innocent" chromosomal aberration [inv(7)(p13q35)] in peripheral blood. Previously we have shown that the frequency of the inv(7) is 100-fold increased in the peripheral blood of patients with ataxia-telangiectasia. These individuals also have an approximately 100-fold increased risk of developing malevolent translocations associated with the development of malignant lymphoma. A population of agriculture workers who demonstrte an increased risk of development of lymphoma also show an increased frequency of the inv(7) with seasonal variation related to their exposure to certain pesticides, fumigants, and herbicides. We have joined in a large scale prospective study of agriculture workers to test the validity and reproducibility of this potential screening test for lymphoma risk. We have recently made a logistic advancement with regard to this study with the refinement of the assay such that it can detect 1-40 copies of the inv(7) from DNA extracted from 200 microliters of peripheral blood spotted on filter disks. This greatly facilitates the acquisition and storage of samples for such large scale studies and may provide a general technique for detecting rare chromosomal aberrations in large population screens. This particular assay may be more than an ancillary marker for the development of cancer-associated chromosomal aberrations because the mechanism that causes the inv(7) (V(D)J recombination) is, at least in part, the same mechanism that mediates many of the leukemia/lymphoma-associated translocations. Selected examples of chromosomal aberrations in which the V(D)J recombinase is likely to have played a role will be discussed.

T 008 TRANSCRIPTION FACTORS AND CHROMOSOMAL TRANSLOCATIONS IN LEUKAEMIA, Terence H. Rabbitts, T. Neil Dear, Paul Fisch, Hirotaka Osada, Isidro Sanchez-Garcia, Viia Valge-Archer and Alan Warren, Medical Research Council Laboratory of Molecular Biology, Cambridge, England.

The development of lymphoid tumours is frequently associated with chromosome abnormalities which result in the activation of oncogenes. In humans, there is a variety of different abnormalities associated with different classes of tumours. We are characterising some genes which contribute to T cell acute (T-ALL). A general pattern has emerged in T-ALL (applicable to acute leukaemia in general) of transcription factors activated by chromosome translocations. Our studies are focussed on the role of two families of gene products in T-ALL.

THE RBTN/Ttg FAMILY OF T CELL ONCOGENES

A common chromosome translocation in childhood T-ALL is t(11;14)(p13;q11) and a more rare one is t(11;14)(p15;q11). Both of these translocations are caused by abnormal rearrangement of the *TCRD/A* locus at 14q11, resulting in the inter-chromosomal joining. Variant forms of the former exist t(7;11)(q35;p13) involving the *TCRB* locus at 7q35 (in collaboration with Kaneko *et al.*, Japan). Two related genes are found at the 11p junctions, and these encode cysteine-rich proteins (LIM domain proteins) with significant homology to iron-sulphur proteins such as ferredoxins. The genes have been called *RBTN1/Ttg-1* (11p15) and *RBTN2/Ttg-2* (11p13). A third member of the family (*RBTN3*) has been cloned and located to 12p12-13. We are studying the rbtn proteins and their possible role in transcription, and are attempting to develop animal models to study normal function and tumour development. Transgenic mice with either *rbtn1* or *rbtn2* expressed in thymus cells develop acute lymphoblastic T cell lymphomas. The frequency is low, however, with about 10% of transgenic animals developing disease, but many of the aspects of the disease are like the childhood ALL from which these genes were first identified.

HOX11, A NEW HOMEOBOX GENE INVOLVED IN T-ALL

We have isolated the *HOX11* gene from junctions of t(10;14)(q24;q11) and t(7;10)(q35;q24) translocations via association with *TCRD* (14q11) and *TCRB* (7q35). The gene encodes a protein with a homeobox and an N-terminal region with features of a transcriptional activation domain. A crucial DNA contacting residue in helix 3 of the HOX11 homeodomain has a threonine residue rather than the usual valine or isoleucine. The role of this residue in DNA recognition is being studied, and we have identified a family of related genes with a threonine at this position, indicating the importance of this substitution. The *HOX11* gene seems to be ectopically activated by the chromosome translocation in childhood T-ALL with such translocations. The studies at present are designed to understand the role of this DNA-binding transcription factor in leukaemia.

Cell Biology and Lymphokines

T 009 MOBILIZATION, PURIFICATION AND IN VITRO EXPANSION OF HUMAN PERIPHERAL BLOOD PROGENITOR CELLS, Roland Mertelsmann, Wolfram Brugger, Lothar Kanz. University of Freiburg, Medical Center, Department of Hematology/Oncology, W-7800 Freiburg, Germany.

As a first step, we have studied the requirements that have to be met to combine an effective cancer chemotherapy with the simultaneous mobilization of PBPCs. It could be shown that there is a differential induction of high numbers of clonogenic PBPCs following standard dose chemotherapy (vepeside, ifosfamide, cis-Platin; VIP-regimen) plus treatment with CSFs (G-CSF; GM-CSF; IL-3 + GM-CSF). The number of CD34-antigen positive cells was highly variable depending on the growth factor used and the prior treatment of the patients. Moreover, mobilized $CD34^+$ cells - depending on the cytokines used for recruitment - had a varying cloning efficiency, and were heterogenous as to their level of commitment. Retransfusion of G-CSF-primed progenitor cells following 3 day high dose chemotherapy demonstrated that PBPCs recruited by standard dose chemotherapy plus G-CSF accelerated both neutrophil and platelet recovery.

In order to enrich for $CD34^+$ cells, peripheral blood mononuclear cell samples were labeled with a biotinylated anti-CD34 monoclonal antibody (12.8) at day 9-12 after chemotherapy, and $CD34^+$ cells were isolated by using an avidin-biotin immunoaffinity column (CellPro, Inc; Bothell, WA, USA). Enriched $CD34^+$ cells were more than 90% pure as determined by morphology and flow cytometry. Their cloning efficiency as analyzed by CFU-GM, BFU-E and CFU-GEMM colony formation was 2.1% (range 0.3 - 7%). Serum containing liquid cultures were performed in microtiter plates for up to 18 days in the presence of different recombinant growth factors or growth factor combinations. Enriched PBPCs were shown to proliferate optimally in the presence of a combination of SCF, IL-1ß, IL-6, IL-3 and Epo. The amplification of total colony forming cells at the day of maximal proliferation was up to 1000-fold (median 290, range 50-930) as compared to baseline values before in vitro expansion. Our data indicate that chemotherapy plus G-CSF primed PBPCs from cancer patients can be expanded in vitro by a combination of hematopoietic growth factors. Large scale expansion of PBPCs harvested from patients could provide high numbers of clonogenic progenitors for use in high-dose chemotherapy and as targets for gene transfer and should facilitate purging of contaminating residual tumor cells.

Therapy

T 010 RECENT ADVANCES IN THERAPY OF HIGH-GRADE T- AND B-CELL NON-HODGKIN'S LYMPHOMAS (NHL) OF CHILDHOOD, Sharon B. Murphy[1], Michael Amylon[2] Michael P. Link[2], W. Paul Bowman[3], Martin Brecher[4], Terry Pick[5], Costan W. Berard[6], Robert Hutchinson[7], and Jonathan J. Shuster[8] for the Pediatric Oncology Group (POG)[9], [1]Chiidren's Memorial Hospital, Chicago, IL 60614, [2]Stanford University, Stanford, [3]Cook Children's Hospital, Fort Worth, [4]Roswell Park Memorial Institute, New York, [5]Brooke Army, Ft. Sam Houston, [6]St. Jude's, Memphis, [7]SUNY, Syracuse, [8]University of Florida, Gainesville, and [9]St. Louis, MO.

We have achieved successive improvements in outcome for all types of pediatric NHL seen over the last decade within the POG, utilizing a stage-and phenotype/histology-specific, risk-adapted approach to treatment.

In successive studies aimed at reducing therapeutic intensity for children with a favorable prognosis, we have achieved a 5 year - event-free survival (EFS) of 85% (SE 4%) and 5 year overall survival of 94% (SE 3%) in a group of 332 patients aged 1-20 with Stage I and II NHL, and we have concluded that nine weeks of chemotherapy without radiotherapy is sufficient treatment for most children with localized NHL.

Children with advanced Stage III or IV NHL receive alternative more intensive therapy based on histology: lymphoblastic (T), small non-cleaved (SNC) cell (B), and large cell (T, B, other). Utilizing a two-year polychemotherapy regimen, we have achieved a 97% complete remission rate and a 60% EFS at four-years for 224 Stage III-IV lymphoblastic NHLs, an outcome superior to that achieved in more than 400 pediatric T-acute lymphoblastic leukemia (ALL) cases treated the same.

For Stage III and IV SNC (B) NHL, and B (SIg+) ALL, we have found that six months treatment with an intensive high-dose chemotherapy regimen (without transplant) is curative for the majority of cases: 76% 2-year EFS for the best arm (Total B Therapy) for Stage III, n=52, 75% for Stage IV n=34 and 60% for B-ALL, n=47 representing a more than three-fold improvement in outcome compared to previous POG results.

Large cell lymphomas (LCL) are heterogenous in both morphology and immunophenotype. Long-term EFS has been achieved in the majority (65-75%) of over 100 Stage III or IV LCL treated with one-year of either ACOP+ or APO therapy (without radiation).

In summary, the majority of children and adolescents with NHL are curable with optimal management. Future research must be directed at further optimization of the risk: benefit ratio of modern multidrug treatment.

T 011 NEW THERAPIES OF HODGKIN'S DISEASE, Saul A. Rosenberg, Stanford University, Stanford.

New therapies of Hodgkin's disease are being directed toward reducing the acute toxicity and long-term morbidity of the highly curative therapies now available. New management programs for patients with favorable highly curable disease reduce the total dose of alkylating agents and other leukemogenic drugs; minimize the long-term effects on cardiac and pulmonary function; and reduce the volume and dose of irradiation so that the incidence of secondary cancers can be reduced. The use of exploratory laparotomy and splenectomy is being limited whenever possible. These new management programs must not reduce the overall cure and survival rates for patients with good prognoses.

For patients with poor prognoses, new dose intensive regimens are being developed with or without adjuvant irradiation. These programs should also be planned so that long-term secondary malignancies and acute leukemia rates should be kept at a minimum. A secondary goal is to reduce the overall incidence of sterility for young patients with a high curative potential.

The proper utilization of autologous bone marrow and/or stem cell support to achieve very high dose intensity of combined modalities should be applied appropriately and whenever possible, compared to secondary or salvage treatment programs with less intensive therapies.

Studies of these types that are underway will be summarized and others proposed.

Controversies-I

T 012 **MULTIDRUG-RESISTANCE IN LYMPHOMAS.** William S. Dalton, Thomas P. Miller, and Thomas M. Grogan, University of Arizona Cancer Center, Tucson, Arizona, 85724.

The problem of drug resistance that eventually develops in patients with lymphomas is one of "acquired" resistance. In other words, drugs that were initially effective in reducing tumor burden become ineffective by selecting for drug-resistant cells over time. Natural product drugs such as the anthracyclines and vinca alkaloids are particularly effective in the treatment of lymphomas. One form of drug resistance that develops to these drugs is termed "multidrug-resistance" or "MDR". MDR is due to the overexpression of an integral membrane protein given the name P-glycoprotein (P-gp) or P-170. P-gp acts as a drug efflux pump which eventually extrudes drugs from cells, thereby preventing a cytotoxic drug from reaching the cellular site of action. Overcoming or preventing this form of drug resistance may represent a new approach to improving treatment outcome.

Immunohistochemical and molecular techniques have been used to measure P-gp in lymphomas. Newly diagnosed and untreated patients rarely express the P-gp in detectable amounts, whereas patients with recurrent and drug refractory disease frequently have detectable levels of P-gp. In a consecutive series of 42 patients with newly diagnosed lymphomas, only one patient expressed P-gp on the malignant cells. In contrast, 7 of 11 patients (64%) who had recurrent, clinically drug-resistant disease expressed P-gp. These studies suggest that the expression of P-gp closely parallels the response to chemotherapy. Similar results have been observed for the B-cell malignancy, multiple myeloma.

Clinical studies to overcome drug resistance due to the overexpression of P-gp have been conducted in patients with drug-resistant malignant lymphoma. Verapamil, a calcium channel blocker known to reverse MDR *in vitro*, was administered as a continuous 5-day infusion with a 4-day infusion of vincristine and doxorubicin. Five complete remissions (28%) and eight partial remissions (44%) were observed among 18 patients studied. These responses may be partially due to the addition of verapamil as well as the administration of drugs by continuous infusion. Both approaches are known to reverse resistance due to MDR *in vitro*.

In vitro studies using cell lines have demonstrated that other mechanisms of drug-resistance exist. Chemosensitizers in combination with doxorubicin select for at least one alternative drug resistance mechanism which is secondary to altered topoisomerase II function. Other membrane proteins besides P-gp may also be involved in drug transport and resistance. These studies indicate that clinical drug resistance is likely to be multi-factorial and multiple approaches to overcoming this problem will be necessary.

T 013 **TREATMENT OF ADVANCED NON-HODGKIN'S LYMPHOMAS**, Richard I. Fisher, Loyola University Medical Center, Maywood, IL. 60153

Therapy for aggressive non-Hodgkin's lymphomas (identified as intermediate and high-grade lymphomas under the Working Formulation Classification) has undergone significant evolution in the last 25 years. Early combination chemotherapy studies with CHOP in the Southwest Oncology Group (SWOG) produced complete response rates (CR) of 50-55% with 30-35% long term survivors. Single institution third generation regimens such as ProMACE-CytaBOM, m-BACOD, and MACOP-B resulted in 68-86% CR with 58-69% survival. Late relapses have been observed in each of these studies. SWOG subsequently conducted a series of Phase II confirmatory trials using the last three regimens. CR varied from 49-65% and survival varied from 50-61%. Since the results from these recent studies are closer to those obtained with the first generation regimens in a national cooperative group setting, ultimate conclusions concerning the efficacy of these new regimens awaited the results of prospective randomized trials.

Between May, 1986 and June, 1991, 1138 previously untreated patients with bulky Stage II, Stage III, or Stage IV, intermediate or high grade, non-Hodgkin's lymphoma were randomized to receive treatment with either standard therapy, CHOP, or one of the third generation chemotherapy regimens, m-BACOD, ProMACE-CytaBOM, or MACOP-B. Each treatment regimen was administered exactly as initially described. Treatment arms are well balanced for patient characteristics. Median follow-up for all patients is 31 months; There is no significant difference in the overall response or complete response rates between treatment arms. At 4 years the percent of patients alive without disease is as follows: CHOP, 36.4%; m-BACOD, 34.4%; ProMACE-CytaBOM, 45.1%; MACOP-B, 38.8% ($p = 0.14$). No difference in overall survival is seen between the treatment arms ($p = 0.67$). Fatal toxicities have been observed in 1% with CHOP, 5% with m-BACOD, 4% with ProMACE-CytaBOM, and 6% with MACOP-B.

Based on these results, new treatment approaches must be developed. We have chosen to focus on two new strategies: preventing the development of the multidrug resistance phenotype and using colony stimulating factors to significantly dose escalate the current treatment regimens.

Controversies-II

T 014 DOES IMMUNE CLASSIFICATION OF LYMPHOMAS MEAN ANYTHING? F. Cabanillas M.D., University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

A major controversy in this field is whether the peripheral T cell lymphomas (PTCL) have a different clinical outcome than the corresponding B cell types. To settle this point it is necessary not only to compare similar histological types but also equivalent stages within each phenotypic category, a point which has been overlooked in most series. An important source of confusion in the literature is the inclusion of cases that nowadays would be recognized as "T cell rich B cell lymphomas" (TCRBCL). In this entity, more than 90% of the cells are of T origin but the remaining 10% which are of B type constitute the malignant clone. In our experience, TCRBCL have a prognosis very similar to the B cell large cell lymphomas (BCLCL). Genotyping is sometimes necessary to identify these cases. None of the published series have included genotyping. With few exceptions, there exists consensus that PTCLs present with more advanced disease. Six series analyze the 3 yr. survival of PTCL and compare it to BCLCL, irrespective of stage. With the exception of the Stanford series, all others show a consistent trend for a lower survival for PTCL. Only the Nebraska series specifically compares the stage IV cases. The survival of stage IV was strikingly poor and significantly worse than for the corresponding B cell cases. This is similar to our experience at MD Anderson Hospital. The Stanford and Tennessee series, also revealed a poor survival for stage IV cases but no direct comparison was available for corresponding cases of BCLCL. In summary: 1- PTCLs appear to present with advanced Ann Arbor stages. 2- stage IV appears to be associated with a remarkably poor outcome, worse than expected for corresponding BCLCL thus suggesting that the T cell phenotype has an influence on prognosis that is independent of their usually advanced presentations. 3- T cell receptor and JH gene rearrangement as well as immunoperoxidase studies should ideally be performed to confirm the cell of origin. The optimal therapy of these disorders is currently unknown.

T 015 WHAT IS MYCOSIS FUNGOIDES?, Richard T. Hoppe, Department of Radiation Oncology, Stanford University, Stanford CA 94305

Mycosis fungoides (MF) was first described by the French dermatologist Alibert more than 150 years ago. The name described the mushroom-like appearance of the cutaneous tumorous lesions, but was not meant to imply a fungal etiology for the disease. More recently, MF was among the first lymphomas which was identified to be of T-cell origin. This placed mycosis fungoides in the general category of diseases termed cutaneous T-cell lymphoma (CTCL), which also includes the Sezary syndrome, lymphomatoid papulosis, and similar disorders.

MF is almost always CD4+/CD8- (helper T-cell phenotype) and usually retains expression of pan-T-cell antigens such as CD2, CD3, and CD5. It is usually Ki-67- and TAC- (a useful distinguishing feature from other adult T-cell lymphomas) and in the majority of cases there is Leu-8 or CD7 deficiency (sometimes helpful for distinguishing from benign inflammatory infiltrates).

Monoclonal T-cell receptor (TCR) gene rearrangements can be demonstrated by Southern blot DNA analysis of the cells derived from cutaneous plaques, tumors, peripheral blood mononuclear cells, or lymph nodes involved by MF. Identical TCR gene rearrangements may be demonstrated from different sites in a single patient, confirming the monoclonal nature of the disease.

A viral etiology for some cases of MF has been suggested. The initial isolation of HTLV-I was from a patient with CTCL, but it was an adult T-cell lymphoma with cutaneous manifestations, not classical MF. Nearly all patients with MF are HTLV-I seronegative. Recently, however, HTLV-I-related DNA sequences have been demonstrated, using PCR techniques, in the peripheral blood mononuclear cells and cutaneous lesions of patients with MF who are seronegative for HTLV-I.

A relationship between the disease, occupational exposure to chemicals and solvents, and chronic antigenic stimulation has been proposed. However, most recent epidemiological studies fail to confirm this hypothesis. The etiology of MF remains undefined.

The natural history of MF has been well defined. There is often a long history of pre-existing skin lesions and the survival of patients may be quite good, even without specific therapy. Cutaneous disease may respond promptly to a variety of treatments, especially ionizing irradiation, topical chemotherapy, and photochemotherapy (PUVA). The development of cutaneous tumors is an unfavorable prognostic sign and may be accompanied by extracutaneous spread of disease, which is often refractory to treatment. The poor results of standard therapies in this setting make the study of investigational therapies such as interferon, monoclonal antibodies, and photopheresis appropriate.

T 016 Early or Late Treatment of Low Grade Lymphomas? **Carol S. Portlock, Memorial Sloan-Kettering, New York.**

Low grade lymphomas are indolent diseases with a long median survival (7 years). Management strategies must take into account such factors as histologic subtype (FML has a lesser frequency of bone marrow involvement and progresses more rapidly if left untreated), stage (although uncommon, stage I presentations may be irradiated with high rates of durable remission), age (autologous stem cell transplantation, ASCT, is only feasible in patients physiologically <50-60 years) and treatment intent (potentially "curative", as in Stage I irradiation or in ASCT approaches; or "palliative", in which disease control alone is the aim and "watchful waiting" may be employed.)

Given the current technology of ASCT, when should patients undergo such intensive therapy? At MSKCC, a prospective study is underway to answer this important question and preliminary data will be presented. Induction chemotherapy achieves CR in 60-75% of all patients with advanced low grade lymphoma. The remainder often have residual bone marrow involvement. Critical to good outcome with ASCT is bone marrow status, arguing for early and elective institution of induction therapy to achieve the highest rate of CR.

Once stem cells are successfully harvested however, it is more difficult to argue that transplantation must be performed in first CR rather than in second remission. First CR is often of good quality, lasting an average 2-4 years; with combined modality regimens, this may extend beyond 5 years. Moreover, initial CR patients have a high likelihood of achieving second CR and later successful outcome with ASCT.

The risks of delaying ASCT to second remission include the possible emergence of histologic transformation, drug resistance or medical contraindications. The advantage to first CRs in delaying intensification (if already harvested), is reduced acute morbidity/mortality and a good quality first remission.

ASCT is certainly indicated in selected patients in first PR, although it is less likely to achieve a durable CR in this setting. The role of upfront ASCT intensification in CR remains a potentially successful curative strategy which requires further prospective study.

Normal B and T Cell Development

T 100 CLINICAL AND PATHOLOGICAL EVOLUTION OF A T-CELL RICH B-CELL LYMPHOMA, Salvatore De Vita, Valli De Re, Riccardo Dolcetti, Antonino Carbone*, Alessandra Marzotto, Umberto Tirelli° and Mauro Boiocchi, Department of Experimental Oncology 1, *Pathology and °Clinical Oncology, Centro di Riferimento Oncologico, Aviano (PN), Italy

We report the biological characterization by immunohistochemical and molecular analysis of multiple tumor biopsies of one patient with an initial hyperplastic lymphadenopathy, which later proved to be a T-cell-rich B-cell lymphoma (TCRBCL), who subsequently developed a diffuse large cell lymphoma . This was a 44-year-old woman with a history of waxing and waning lymph node enlargment and an indolent clinical course during a period of 3 years: temporary complete clinical regression was observed concomitantly with repetead courses of low-dose corticosteroid therapy. Immunogenotypic analysis of 4 consecutive biopsies during this period revealed that the same B-cell clone, showing the unusual presence of a IgK gene rearrangement and the heavy-chain gene in germline configuration, was invariantly present in all the phases of the disease, intermingled with an overwhelming amount of polyclonal T-cells. Subsequent to chemotherapic and radiation therapy, an additional IgH rearrangement occurred and a diffuse large cell (large non-cleaved or centroblastic) B-cell lymphoma developed.

TCRBCLs present strong analogies and may resemble, at least in the initial phases, the follicular center cell lymphomas of SJL mice, in which two cellular populations are reciprocally dependent for growth (clonal B-cells recruiting polyclonal T-cells, which are non-cytotoxic, but lymphokine-secreting to sustain B-cell growth). Such a biological loop could favour the occurence of further genetic alterations in the clonal population and the malignant monomorphous progression of the disease.

T 101 FACTS AND FICTION IN HODGKIN'S DISEASE. A NEW TECHNIQUE TO CHARACTERIZE TUMOR CELLS.

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Diverse data suggest that in Hodgkin's disease also bystanding cells might be involved in clonal proliferation. This is supported by the frequent finding of non-clonally aberrant cells in addition to a clone in cytogenetic analysis. These non-clonal cells can neither be investigated concerning putative clonality due to the low number of available mitoses nor can they be affiliated to a certain cell lineage. For this reason we have developed a method to directly combine fluorescence immunophenotyping and interphase cytogenetics. This technique is named "Fluorescence-immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms (FICTION)". We have employed FICTION in a case of Hodgkin's disease exhibiting a complexly aberrant major clone and an additional minor clone with solely trisomy X. Using FICTION we could show that the trisomy X cells are CD3 positive T-cells, while the major clone represents CD30 positive, CD3 negative HRS-cells.

T 102 HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR PEDIATRIC PATIENTS WITH RELAPSED NON-HODGKIN AND HODGKIN LYMPHOMA: USE OF THIOTEPA-BASED PREPARATIVE REGIMENS. Bruce Gordon, Minnie Abromowitch, Phyllis Warkentin, Sarah Strandjord, Peter Coccia. Dept of Pediatrics, University of Nebraska Medical Center, Omaha, NE 68198

HSCT is effective therapy for patients with relapsed non-Hodgkin lymphoma (NHL) and Hodgkin disease (HD). We have used high dose thioTEPA (TT), a polyfunctional alkylating agent, in combination with other agents, followed by autologous (auto) or allogeneic (allo) HSCT, to treat 24 children (2.6-17.8 yrs) with relapsed NHL and HD. 15 pts underwent HSCT for large cell lymphoma (LCL; 11 T cell lymphoma, 4 non-T cell lymphoma), 5 pts for high grade NHL (4 small non-cleaved lymphoma [SNCL], 1 lymphoblastic lymphoma [LL]) and 4 pts for HD. Regimens used were TT 300 mg/m^2 QD X 3 days followed by TBI 300 cGy BID X 3 days (TT/TBI); VP-16 1000-1800 mg/m^2 X 1, followed by TT/TBI (VP/TT/TBI); and VP/TT followed by cyclophosphamide 50 mg/kg QD X 4 (VP/TT/CY). 15 pts underwent auto (n=11) or allo (n=4) HSCT for LCL using TT/TBI (n=2) or VP/TT/TBI (n=13). 10 of these 15 are alive without disease (9 of 11 T cell and 1 of 4 non-T cell) at median 24 mos (range 6-52 mos) with RFS 66%. 3 of 15 pts relapsed at a median of 1 mo after HSCT (range 1-5), 1 additional pt died early of sepsis and 1 died in remission 9 mos after HSCT. 5 pts underwent auto (n=3) or allo (n=2) HSCT for SNCL or LL using VP/TT/TBI. All 5 relapsed at median 2 mos after HSCT (range 1-4 mos). 4 pts underwent auto (n=3) or allo (n=1) HSCT for HD using VP/TT/CY. 2 of these 4 pts are alive without disease 6+ and 44+ mos after HSCT. The other 2 pts relapsed at 4 and 29 mos after HSCT. Toxicity of the TT containing regimens was significant, but limited primarily to severe oropharyngeal mucositis and generalized erythroderma. Based on these results, we conclude that HSCT following TT/TBI ± VP-16 is effective salvage therapy in children with relapsed LCL, especially T cell LCL. There is no evidence from our small study that TT containing regimens are effective for pts with SNCL. Further evaluation of these regimens are ongoing for LCL, HD and LL.

T 103 DEPLETION OF B CELLS *in vivo* BY A CHIMERIC MOUSE HUMAN MONOCLONAL ANTIBODY TO CD20, Mitchell Reff, John Leonard, Roland Newman, Nabil Hanna and Darrell Anderson, IDEC Pharmaceuticals Corporation, La Jolla, California 92037

Murine monoclonal antibody 2B8 specifically recognizes the CD20 phosphoprotein expressed on the surface of normal B lymphocytes and B cell lymphomas. The light and heavy chain variable regions of 2B8 were cloned, after amplification by the polymerase chain reaction, into a cDNA expression vector, which contains human IgG1 heavy chain and human kappa light chain constant regions. High level expression of chimeric 2B8 antibody (C2B8) was obtained in both mouse myeloma cells (SP2/0) and Chinese hamster ovary (CHO) cells. Purified C2B8 exhibited antigen binding affinity and human tissue reactivity similar to the native murine antibody. *In vitro* studies demonstrated the ability of C2B8 to bind human C1q, mediate complement dependant cell lysis (CDC) of human B lymphoid cell lines, and lyse human target cells through antibody dependant cellular cytotoxicity (ADCC). Infusion of macaque cynomolgus monkeys with doses ranging from 1.6 mg/kg to 10 mg/kg resulted in >98% depletion of peripheral blood B cells and 40 to 70% depletion of lymph node B cells. Recovery of peripheral blood B cells usually started at two weeks after treatment and required 60 to greater than 90 days to reach normal levels. A 95% depletion of B cells in peripheral lymph nodes and bone marrow was observed in a high dose pharmacology study. No toxicity was observed in any of the animals. These results offer the possibility of using an "immunologically active" chimeric anti-CD20 antibody as an alternative approach to the treatment of B cell lymphoma.

T 104 A NOVEL HUMAN CELL LINE (DEGLIS) WITH DUAL B:T PHENOTYPE AND GENE REARRANGEMENTS AND CONTAINING EPSTEIN-BARR VIRUS GENOMES, B.Rubin, T.A.Saati, H.J.Delecluze, S.Chittal, P.Brousset, J.P.Magaud, N.Dastugue, E.Cohen-Knafo, G.Laurent and G.Delsol. CRPG/CNRS, CHU de PURPAN, Toulouse, France.
A new cell line termed Deglis was established from a polymorphic centroblastic lymphoma. The cell line and its source carry a dual B-cell and T-cell phenotype and Epstein-Barr virus (EBV) genomes. Simultaneous expression of B-cell (CD19, CD20, CD23, CD37) and T-cell (CD2, CD7, CD43) antigens, activation antigens (CD30, CDw70) as well as CD68, a macrophage-associated antigen, was observed . Genotypic studies of the cell line showed dual gene rearrangements: Jh and Ck were rearranged without expression of cytoplasmic or surface immunoglobulin. T-cell receptor (Tcr)-a and Tcr-b genes were rearranged, whereas Tcr-g and Tcr-d genes were in germline configuration. Apparently, functional transcripts of Tcr-a and truncated transcripts for Tcr-b and Tcr-d were observed. CD3-g, CD3-d, and CD3-z mRNA were present but CD3-e mRNA was not detected. EBV-encoded proteins (LMP and EBNA2) were expressed . Southern blot analysis showed the same clonal EBV genomes in the primary tumor and the cell line. Karyotypic analysis of the cell line showed several chromosomal abnormalities but normal chromosome number. The characteristics of this cell line suggest that neoplastic transformation has occured in a precursor cell broadly committed to lymphoid lineage. Studies are in progress in order to verify whether the Deglis Tcr-a chain is functional (sequence analysis) and understand why there is no CD3-e transcripts. This cell may help to resolve some issues in the physiopathology of lymphoid tumors and in complex biologic phenomena such as Tcr/CD3 biosynthesis, assembly and membrane expression.

T 105 CYTOGENETIC FINDINGS IN 104 PERIPHERAL T CELL LYMPHOMAS CORRELATE WITH HISTOPATHOLOGICAL DIAGNOSES OF THE UPDATED KIEL CLASSIFICATION. Brigitte Schlegelberger, Annekathrin Himmler, Werner Grote, Alfred C. Feller°, Karl Lennert*. Institute of Human Genetics, University of Kiel, Institutes of Pathology, University of Lübeck° and Kiel*, Germany.
Cytogenetic studies were performed on lymph node biopsies, skin biopsies and peripheral blood from 104 patients with peripheral T cell lymphomas (PTL). The diagnoses were made according to the updated Kiel classification. Low grade PTL showed consistent cytogenetic features. Clones with inv(14) and trisomy 8q were found in all cases of T-CLL and T-PLL. The distal breakpoint of *clonal* inv(14) lay in 14q32.1 and differed from the distal breakpoint of *nonclonal* inv(14). It is supposed that band 14q32.1 contains an oncogene that is essential for the tumor development in T-CLL and T-PLL. AILD-type PTL was characterized by +3, +5 and +X clones. Trisomies 3 and 5 were also observed in T-zone and lymphoepitheloid lymphomas. However, different single cell aberrations and unrelated clones were exclusively detected in AILD-type PTL and appeared in 30 out of 50 cases. In the high grade PTL series only LCAL showed a characteristic chromosome aberration, i.e. t(2;5)(p23;q35). The other high grade PTL contained in contrast to low grade PTL usually complex clones with many structural aberrations and often chromosome numbers in the triploid to tetraploid range. Recurrent aberrations of medium to large cell pleomorphic lymphomas were dup(6)(p12-p21/2), del(6)(q15q25), total or partial trisomy 7q and breaks in 13q14. Transition from low grade to highgrade PTL was repeatedly accompanied by deletions in 1p, indicating a short survival. In summary, the cytogenetic findings in our series of 104 PTL paralleled the histopathological diagnoses of the updated Kiel classification. Thus, cytogenetic findings can be helpful to clarify uncertain cases of PTL.

T 106 BCL-3 GENE EXPRESSION IN HUMAN B CELLS IS UPREGULATED BY EPSTEIN-BARR VIRUS INFECTION. William Scouten and Brian A. Pollok, Department of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, NC 27157.
Human B cell lines without apparent rearrangement in the bcl-3 locus were analyzed for steady-state bcl-3 mRNA levels by Northern blotting. Very low amounts of mature bcl-3 transcripts were expressed among B cell lines negative for EBV. This low baseline level for bcl-3 mRNA expression was also observed among EBV-positive B cell lines displaying the limited group I (EBNA1 only) or group II (EBNA1 and LMPs) patterns of EBV latent gene expression. A dramatic upregulation of bcl-3 mRNA levels was seen among B cell lines which possess the group III EBV phenotype where all ten EBV latent genes are expressed. Both group III Burkitt lymphoma lines and karyotypically-normal lymphoblastoid cell lines experienced 4 to 20 fold higher bcl-3 mRNA levels as compared to group I/II B cell lines of similar origin. Group III Burkitt lymphoma lines carrying either EBV-1 and EBV-2 serotypes were found to have elevated bcl-3 expression. Conversion of an EBV-negative Burkitt lymphoma by EBV infection in vitro produced a ten-fold increase in bcl-3 expression. Stable transfection of EBV-negative and EBNA2-defective Burkitt lymphoma cell lines with an EBNA2 expression vector failed to activate bcl-3 expression. Current work is focused on defining the timing of bcl-3 upregulation during EBV infection and which EBV gene product is responsible.

T 107 THE ORIGIN OF $CD8^+$ ALLO-CLASS II MHC SPECIFIC CTL. Nobukata Shinohara*, Koji Eshima*, Minesuke Yokoyama#, Motoya Katsuki# and Harumi Suzuki#, *Mitsubishi Kasei Insitute of Life Sciences, Tokyo, Japan, and #Central Laboratoey of Experimental Animals, Kawasaki, Japan
$CD8^+$ CTLs constitute a large portion of immune responses to allogeneic class II MHC antigens. In an attempt to elucidate the origin of such T cells with a discordant combination of co-receptor expression and MHC-specificity, we developed transgenic mice bearing TcR α and β chain genes of a $CD8^+$ allogeneic class II MHC ($I\text{-}A^+$) specific CTL clone QM11. In transgenic mice with the H-2 haplotype of the original B10.QBR (bbbq) strain, cells expressing high density of the QM11 idiotype ($ID11^{high}$) matured into $CD8^+$ and double negative (DN) populations, both of which were readily reactive to antigenic stimumation with $I\text{-}A^k$-bearing cells. In B6 (bbbb) transgenic mice, $ID11^{high}$ cells did not mature into $CD8^+$ cells indicating that they were positively selected by D^q or L^q. This result was corroborated by effective maturation of $CD8^+$ $ID11^{high}$ cells in B10.SQR mice which shared only D^q and L^q with B10.QBR in common. In contrast, $ID11^{high}$DN cells matured even in B6 mice, suggesting the absence of positive selection during their ontogeny. Such cells might be transgenic artefact developed due to premature expression of transgenic TcR genes. In mice expressing the target antigen, $I\text{-}A^k$, $ID11^{high}$ cells of both types were completely depleted. Taken collec-tively the results indicate that a $CD8^+$ allo-class II-specific CTL clone QM11 belongs to the conventional $CD8^+$ cell population maturing through positive selection by class I MHC antigens.

T 108 **CLONAL T-CELL RECEPTOR GAMMA CHAIN VARIABLE GENES EXPRESSED IN ADULT T-CELL LEUKEMIA.** **Ahmet Z. Uluer, Phyllis M. Overturf, Michael H. Rickert, Steven J. Greenberg, Department of Neurology, Roswell Park Cancer Institute, Buffalo, NY 14263.**

The somatic rearrangement of the T-cell antigen receptor (TCR) provides for a novel approach to identify leukemic clones *ex vivo* from unmanipulated samples of blood, marrow, cerebrospinal fluid, or from any lymphoid-bearing tissue specimen. A genomic DNA-based gene amplification strategy was developed to molecularly define whether the TCR-gamma gene effectively rearranges, as expected, in adult T-cell leukemic (ATL) α/β expressing clones and whether there was restricted heterogeneity among the TCR-γV repertoires clonally rearranged. By this method, PCR was anchored by a generic primer to a relatively conserved TCR-γJ region and complemented with a generic TCR-γV primer that was composed of a combination of γV degenerate and γV family-specific primers. A PCR directed in this manner was capable of universal amplification of all rearranged TCR-γVJ clonotypes. Aliquots of amplicons were thereafter liquid hybridized with family-specific TCR-γV radiolabeled internal probes, subjected to gel retardation electrophoresis, and autoradiographed. Photographs were scanned densitometrically and the TCR-γV repertoire profiled. In this manner, the predominant TCR-γV repertoire, corresponding to the T-cell leukemic clone of interest was identified.

T 109 IMMUNOGLOBULIN LIGHT CHAIN DIVERSITY WITHIN A SINGLE TUMOR CLONE IN A FOLLICULAR/CENTROBLASTIC-CENTROCYTIC (CB-CC) LYMPHOMA

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Recently we have developed a technique to detect clonal tumor cells with numerical chromosome aberrations on cytospins and to characterize these individual cells by immunophenotyping. Using this method which combines fluorescence immunophenotyping and interphase cytogenetics we have investigated five cases of follicular/(cb-cc) lymphoma concerning immunoglobulin light chain diversity. All lymphomas had numerical chromosome aberrations in addition to the typical t(14;18)(q32;q21). One of these cases had trisomy 8, and histopathological evaluation had revealed kappa light chain restriction. Using combined fluorescence immunophenotyping and interphase cytogenetics we could prove that in this case besides a major kappa positive population a lambda positive subpopulation coexists within the same tumor clone defined by trisomy 8. We conclude that (a) numerical chromosome aberrations, other than commonly supposed, may occur very early in the development of follicular/cb-cc lymphomas, i.e. prior to rearrangements of the immunoglobulin light chain genes, and that (b) these secondary events might play an as yet underestimated role in early tumor evolution.

T 110 SOMATIC DIVERSIFICATION OF VL BY GENE CONVERSION IN A HUMAN LYMPHOMA, Andrew D. Zelenetz, Ronald Levy, and Yvonne Remache, Division of Hematologic Oncology and Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, NY 10021 and Department of Medicine, Division of Oncology, Stanford Medical School, Stanford, CA 94305.

Human follicular lymphoma (FL) is an indolent disease characterized by monoclonal expansion of mature B lymphocytes. In at least 90% of cases, a chromosomal translocation, t(14;18), can be identified in tumor cells which juxtaposes the *bcl-2* proto-oncogene and the immunoglobulin J_H locus. The molecular anatomy of the translocation breakpoint suggests that the translocation occurs as an error of D-J joining, hence likely arises in the pre-B cell. Thus, follicular lymphomagenesis must involve additional steps beyond the t(14;18) translocation. FL arises from a cell in which somatic mutation of the immunoglobulin variable (V) genes is actively ongoing; hence, there is intra-clonal variation of immunoglobulin V gene sequences. We have recently reported (J. Exp. Med. 176:1137, 1992) an analysis of the pattern of mutation in the rearranged VH gene derived from the tumor relative to a molecular clone of the unmutated germline V gene segment. The results demonstrated that the FL arose after the initiation of somatic mutation. In addition, the pattern of mutation suggested the tumor clone had expanded subsequent to antigenic selection. We have recently extended this analysis to the VL of the same patient and have confirmed the finding of clonal expansion subsequent to antigenic selection. Furthermore, rare molecular clones of the rearranged VL in the FL population demonstrated a 33 bp insertion in CDR1. This insertion was also universally found in clones of the rearanged VL from the transformed diffuse large cell lymphoma (tDL) which arose from the FL in the same patient. The insertion was compatible with immunoglobulin expression since the tDL expressed a surface immunoglobulin. The upstream regions of molecular clones of the VLs derived from the FL and tDL were identical arguing against variable gene replacement as the mechanism of the alteration. Using an oligonucleotide probe derived from the sequence of the CDR1 insertion in Southern blot hybridization of germline, FL and tDL DNA, we specifically identified the rearranged VL allele of the tDL and a second fragment common to all three DNA samples. These findings suggest that the insertion arose via a gene conversion event. Efforts are underway to clone the potential donor sequences identified with the CDR1 specific clone. It is possible that gene conversion represents a mechanism of somatic diversification in human B cells.

T 111 A DEVELOPMENTAL PATHWAY INVOLVING FIVE SUBSETS OF FUNCTIONALLY DISTINCT CD3-CD4-CD8- TRIPLE NEGATIVE ADULT MOUSE THYMOCYTES DEFINED BY CD44 AND CD25 EXPRESSION. Albert Zlotnik, Jacqueline Kennedy, Takashi Suda and Dale I. Godfrey. Department of Immunology, DNAX Research Institute, Palo Alto, CA 94304

We have subdivided mouse $CD4^-CD8^-CD3^-$ triple negative (TN) thymocytes into five subsets based upon expression of CD44 and CD25, including $CD44^+CD25^-$, $CD44^+CD25^+$, $CD44^-CD25^+$, $CD44^-CD25^{lo}$ and $CD44^-CD25^-$. Characterization of these cells revealed distinct features of each individual subset, in particular the expression of c-kit (the receptor for stem cell factor (SCF)) by $CD44^+CD25^+$TN but not by $CD44^-CD25^+$TN. The former subset also responds to IL7 and SCF, whereas only minimal responsiveness was observed by the $CD44^-$ populations. These subsets also showed differential cytokine production potential ($CD44^+CD25^-$ > $CD44^+CD25^+$ > $CD44^-CD25^+$ > $CD44^-CD25^{lo}$ > $CD44^-CD25^-$) following stimulation with calcium ionophore, PMA and IL1. $CD44^-CD25^-$ spontaneously became $CD4^+CD8^+$ after 24 h in culture, while the $CD44^-CD25^+$ cells did not. $CD44^-CD25^{lo}$ cells also spontaneously developed into $CD4^+CD8^+$ cells, although to a lesser extent than $CD44^-CD25^-$TN. These results support the following maturation sequence: $CD44^+CD25^- \rightarrow CD44^+CD25^+ \rightarrow CD44^-CD25^+ \rightarrow CD44^-CD25^{lo} \rightarrow CD44^-CD25^-$. This progression from $CD44^+CD25^+$ to $CD44^-CD25^+$ cells was confirmed by their TCR β-chain gene configuration. The former population exhibits germline TCR β-chain configuration, whereas the latter subset shows a rearranged pattern.

Etiology; Virology

T 200 INFECTIVITY OF A DELETED MOLECULAR CLONE OF HUMAN T-CELL LEUKEMIA VIRUS TYPE II. Gary L. Cockerell[1], Joel Rovnak[1], Patrick Green[2], and Irvin S. Y. Chen[3]. [1]Department of Pathology, Colorado State University, Ft. Collins, CO 80523; [2]Department of Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232; and [3]Departments of Microbiology and Immunology, and Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The proximal portion of the X region of the human T cell leukemia virus (HTLV) family contains a highly conserved region which is not known to be expressed. To determine the role of this region, a molecular clone containing a 324 base pair deletion (nt 6660-6984) was derived from a full length clone of HTLV type II (HTLV-II). The infectivity of the deleted and full length clones was tested following transfection and isolation of human B-lymphoblastoid producer cell lines. There was no difference in the ability of the deleted and full length clones to transform primary human peripheral blood lymphocytes, or to induce syncytia in susceptible target cells as determined by coculture assays. Both the full length and deleted clones were also infectious *in vivo*, as determined by intravenous inoculation of weanling rabbits with lethally irradiated producer cells. There was no difference in the onset or pattern of anti-viral antibody seroreactivity, as determined by western immunoblots, in rabbits inoculated with either full length or deleted clones. Peripheral blood mononuclear cells and other lymphoid cells from rabbits inoculated with either clone produced minimal p24 *in vitro*. Virus was more readily detected by PCR amplification of HTLV-II *tax/rex* sequences. The frequency, distribution and intensity of proviral sequences in tissues was much greater in rabbits inoculated with the full length clone as compared to the deleted clone. No clinicopathological evidence of disease occurred in rabbits observed as long as 24 weeks post-inoculation with either clone. The function of and the reason for the conservation of the proximal X region of HTLV-II are unknown. The results of this study demonstrate that molecular clones with or without this sequence are infectious *in vitro* and *in vivo*. However, this sequence appears to play a role in the accumulation and distribution of provirus in tissues *in vivo*.

T 201 **HUMAN PAPILLOMAVIRUS RECOGNITION BY CYTOTOXIC T-LYMPHOCYTES IN PATIENTS WITH CERVICAL DYSPLASIA AND HPV-16 INFECTION**
B.H.McGovern, E.Androphy, J.Lieberman, Tufts New England Medical Center, Division of Hematology-Oncology and Division of Dermatology, Boston, MA

Human papillomaviruses are a heterogeneous group of DNA viruses associated with proliferative lesions of cutaneous and genital epithelium. Epidemiologic evidence implies a role for cell-mediated immunity in the prevention of HPV-related disease. Female renal transplant patients have a seven-fold increased risk of developing cervical dysplasia and cervical cancer when compared to non-immunosuppressed patients. HIV-infection is associated with increased rates of cervical dysplasia, rapid disease progression to carcinoma, and refractoriness to therapy. Patients at highest risk for cervical dysplasia are those with a diagnosis of AIDS and CD4 counts less than 200.

We plan to use recombinant vaccinia viruses which encode each of the open reading frames of the HPV-16 genome to study the human cytotoxic T-cell response to HPV. PAP smears and peripheral blood will be obtained from patients with abnormal cervical histology. The presence of HPV-16 DNA will be documented using the polymerase chain reaction method. HPV-specific CTL lines will be generated from peripheral blood mononuclear cells and possibly cervical biopsy specimens. Specific lysis of target cells infected with HPV-vaccinia recombinants expressing HPV-gene products will be compared with lysis of vaccinia-lacZ control target cells using a four-hour chromium release assay. We will thus be able to identify which HPV proteins encode antigens recognized by the CTLs of infected patients.

T 202 **Potential Involvement of HEB, a Helix-Loop-Helix Transcription Factor, in T Cell Development.** Jing-Shan Hu, Lawrence A. Turka[1], Craig B. Thompson[1], and Robert E. Kingston Harvard Medical School and Massachusetts General Hospital. [1]University of Michigan.

Proteins containing the helix-loop-helix (HLH) domain have been shown to be important in regulating cellular growth and differentiation. We have isolated a cDNA for a human HLH factor, designated HEB. HEB shares strong similarities in the HLH domains with E proteins encoded by the E2A and ITF2 genes that are involved in regulation of muscle development by forming hetero-dimer with myogenic regulatory HLH proteins (e.g. myogenin and myoD). We have studied the involvement of HEB in regulation of T cell development. We have shown by Northern hybridization analysis and in situ hybridization analysis that the expression of HEB mRNA in thymocytes is developmentally regulated. Our studies further indicate that HEB can bind to regulatory elements present in a T cell-specific enhancer. These elements were shown previously to convey T cell-specific transactivation and bind to T cell-specific nuclear factors. HEB is capable of transactivating gene expression via these elements. When forming the hetero-dimer with a lineage-specific HLH factor (myogenin, a myogenic regulatory factor), HEB can bind to these elements with a specificity similar to that of T cell-specific nuclear factors. Together, these studies suggest that HEB is likely to be involved in cell-type specification in the thymus by transactivating T cell-specific gene expression, perhaps as a hetero-dimer.

T 203 CHROMOSOME ABNORMALITIES IN THE LEUKEMIC PHASE OF NON-HODGKIN'S LYMPHOMA. MT Khokhar, V Brito-Babapulle, E Matutes and D Catovsky. Academic Department of Haematology & Cytogenetics, The Institute of Cancer Research, Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, UK

The leukemic phase of non-Hodgkin's lymphoma (NHL) presents a clinical and diagnostic challenge as these cases present with a high white blood cell count with abnormal lymphoid cells resembling chronic lymphocytic leukemia (CLL). Histology, immunophenotyping and morphologic studies aid in the characterisation of the leukemic phase of follicular (FL), intermediate/mantle cell and lymphoplasmacytic (LPL) types of NHL and in their distinction from B-cell CLL. Specific chromosome translocations have been shown to correlate with some subtypes of NHL, e.g. t(14;18) in FL and t(11;14) in mantle cell lymphoma. We have performed cytogenetic studies in a series of 12 patients with various forms of NHL in leukemic phase to determine: a) whether they can be identified by their specific cytogenetic abnormalities and b) whether additional karyotypic changes are associated with the development of leukemia in NHL. The cases studied included 3 FL, 3 mantle cell lymphoma and 6 LPL. All the cases had marked absolute lymphocytosis (range 15.6-596x10^9/l) and were of B-cell type by immunophenotyping. The material studied included blood (10), bone marrow (1) and spleen (1). The translocation t(14;18)(q32;q21) was demonstrated in 2 cases of FL, t(11;14)(q13;q32) in 2 cases of mantle cell NHL and trisomy 12 or t(12;14)(q13;q32) in 5 cases of LPL. Eleven of the 12 cases had additional chromosome abnormalities, chiefly del(6q) in FL, abnormalities of 8q24 in mantle cell lymphoma and abnormal chromosome 3 in LPL. Overall, 7 cases exhibited chromosome rearrangements not previously reported. Our study demonstrates that: 1) the specific chromosome translocations of NHL subtypes are also demonstrable in the leukemic phase, and 2) additional abnormalities are found in the majority of cases and these may be uniquely associated with leukemic transformation.

T 204CHARACTERIZATION OF HUMAN B CELL LINES DISPLAYING A STABLE EBV LATENCY II PHENOTYPE. Kathleen A. Taylor, Suzanne M. Wetzel and Brian A. Pollok, Dept. of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, NC 27157-1064

Three distinct patterns of latent gene expression have been described for human cells infected with Epstein-Barr virus. All EBV-infected B cells express EBNA1, whereas the other EBNAs and the latent membrane proteins LMP1, LMP2a and LMP2b are only expressed in lymphoblastoid cell lines and Burkitt lymphoma cell lines of the latency III phenotype. This EBV latency stage is typified by transcription of EBNA genes from the C/W promoter region, whereas the latency I or EBNA1-restricted phenotype, seen in newly isolated BL cells, expresses the EBNA1 gene product exclusively from the downstream F promoter. The recently defined latency II phenotype observed in nasopharengeal carcinoma cells exhibits an unusual pattern of latent gene expression; the latent membrane proteins are expressed and EBNA1 is transcribed from the F region promoter without attendant expression of the other EBNA genes. Here we characterize several stable human B cell lines, derived from non-malignant sources, which also display a latency II EBV phenotype. These lat II B cells stabily express LMP1 and LMP2b as well as EBNA1 mRNA and protein. Expression of very low levels of EBNA2 and EBNA3C mRNA could be detected yet the expression of EBNA2 protein was not observed. PCR analysis shows that there is dual promoter usage for the expression of EBNA1 in the lat II B cell lines. The majority of EBNA1 transcripts are generated from the F promoter, with a low level of the transcripts originating from the W promoter. Preliminary experiments on the regulation of EBV latency in the lat II B cell lines demonstrate that the repression in EBNA latent gene expression is neither dominant nor irreversible. Our results show that the latency II phenotype can be stably maintained in B cells in vitro and provides a model for further study of the molecular basis of the latency II phenotype in vivo.

T 205 REGULATION OF PHOSPHOLIPID METABOLISM IN LYMPHOMA , Min Tian and Ruth M. Dixon, MRC Biochemical and Clinical Magnetic Resonance Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K.,OX1 3QU

Introduction : Previous studies from this laboratory have showed an increased phosphomonoester (PME)/ATP ratio in the ^{31}P MR spectra of the livers of patients with hepatic lymphoma. We found similar results in lymphomatous mice, which indicated that the increase in PME was largely due to phosphoethanolamine, while phosphocholine was unchanged. These compounds are intermediates of phospholipid metabolism. We have also shown that radiolabelled ethanolamine is incorporated less rapidly into phosphatidylethanolamine (ptdE) in the lymphomatous liver than in the normal liver. We have now measured the activities of choline kinase (CK), ethanolamine kinase (EK), CTP-choline transferase (CTP-CT) and CTP-ethanolamine transferase (CTP-ET), which are thought to be key enzymes in the regulation of phospholipid metabolism.

Methods: Mice were injected i.v. with A120 lymphoma cells(10^5).After 14 days, when the liver was about 60-70% infiltrated with lymphoma cells, the livers were excised, homogenized and centrifuged. CK, EK and CTP-ET activities were determined in cytosolic fraction. Since CTP-CT has two forms, cytosolic form and microsomal form, this enzyme was measured in both cytosolic and microsomal fractions. Enzyme activities are expressed as IU/mg protein (mean$\pm$ s.d., n=4).

Results : The CK activities were not significantly different between control and lymphoma(26.3 $\pm$2.8 and 22.6 $\pm$ 3.5, respectively). The EK activities were decreased in lymphoma(28.9 $\pm$ 4.2) compared with the control(38.6 $\pm$3.2, $p<0.05$).The cytosolic CTP-CT activity was significantly reduced in lymphoma(0.18 $\pm$ 0.05) compared with the control(0.43$\pm$0.15, $p<0.02$), while the microsomal CTP-CT in lymphoma (0.40$\pm$ 0.12) was almost as the same as that in control (0.43$\pm$0.10). The CTP-ET activity was reduced in lymphoma(2.43 $\pm$ 0.63) compared with the control(3.63$\pm$ 0.59, $p<0.05$). These data show that hepatic phospholipid metabolism is changed following lymphomatous infiltration. We found decreased enzyme activities in the phospholipid synthetic pathways in the lymphomatous liver compared to control, which are consistent with ^{31}P-NMR and radiolabelling studies from this laboratory. These results suggest that the low rate of ptdE synthesis in A120 lymphoma could be due to low EK and CTP-ET activities, although other regulatory mechanisms are not excluded.

T 206 EXPRESSION AND RELEASE OF CD 27 IN HUMAN B CELL MALIGNANCIES. Rien H.J. van Oers, Ludo Evers, Rene A.W. van Lier and Steven T. Pals. Department of Haematology and Pathology, Academic Medical Center, Amsterdam and Central Laboratory of the Red Cross Blood Transfusion Service,Amsterdam,The Netherlands.

Since its original description the transmembrane disulfide-linked 55 kD molecule CD 27 has been considered to be a T cell-specific differentiation antigen expressed on mature thymocytes and the vast majority of peripheral blood T cells. A soluble 28-32 kD form of CD 27 (sCD 27)with extensive structural homology to the 55kD transmembrane molecule can be detected in the supernatant of activated T cells as well as in body fluids like serum,urine and spinal fluid. Recently however, Maurer *et al.* described CD 27 to be present on a subset of normal B lymphocytes in tonsils and peripheral blood, but not in cord blood. Immunophenotypical and functional studies suggested that CD27 expression might be a marker of later stages of B cell maturation. We have studied the expression of CD27 on normal lymphoid tissue and on human malignant B cells representative for several differentiation stages. Significant numbers of CD27+ B cells were only found in the follicle center, notably in the more apical zones, and in the peripheral blood (25+10%). CD27 was not present on malignant cells corresponding to early stages of antigen-independent B cell differentiation. Strong expression was found on CLL, Hairy cells, PLL and low grade malignant follicular non-Hodgkin's Lymphomas. Moreover, using a sensitive sandwich-ELISA we could demonstrate the presence of sometimes massive amounts of soluble CD27 in the sera of patients with B cell malignancies. Both in transversal and longitudinal studies we found a strong correlation between sCD27-levels in serum and disease activity. The functional significance of our findings will be discussed.

Molecular Biology

T 300 **RAPID AND SENSITIVE MOLECULAR GENETIC IDENTIFICATION OF T-CELL RECEPTOR CLONOTYPES *EX VIVO*, Teri F. Beers, Youngnim Choi, Tian-Long Du, Michael H. Rickert, Phyllis M. Overturf and Steven J. Greenberg, Department of Neurology, Roswell Park Cancer Institute, Buffalo, NY 14263**

A novel genomic DNA-based gene amplification strategy was employed to molecularly identify the T-cell receptor beta chain (TCR-ß) variable/diverse/joining (VDJ) regions that defined *ex vivo* an array of unique leukemic clonotypes from adult T-cell leukemia, Sezary Syndrome, and large granular lymphocytosis. By a sequence of tandem gene amplifications, the family-specific variable gene usage of TCR-ß is first identified in a primary PCR which is directed by a TCR-ßJ generic and a complement of family specific TCR-ßV primers. Subsequently, the genetic sequence of the primary amplicon is analyzed to reveal the third complementarity determining region (CDR 3) comprising the juxtaposed VDJ domain. This information is used to generate a unique clonotypic primer that, in a second round of PCR, directs the specific amplification of the unique leukemic clone. By this approach an unparalleled level of sensitivity and specificity is achieved which can be applied to the detection of minimal residual leukemia and for the evaluation of chemotherapeutic efficacy.

T 301 ***EX VIVO* MOLECULAR GENETIC IDENTIFICATION OF RESIDUAL HEMATOPOIETIC MALIGNANT CLONES IN ACUTE LYMPHOCYTIC LEUKEMIA, Youngnim Choi, Tian-Long Du, Phyllis M. Overturf, Michael H. Rickert, and Steven J. Greenberg, Department of Neurology, Roswell Park Cancer Institute, Buffalo, NY 14263.**

A major issue related to autologous bone marrow transplantation (AuBMT) is the ability to determine whether there is inadvertent transplantation of marrow contaminated with residual leukemia. If elimination of residual leukemia is critical to the success of AuBMT, then persistence of leukemia would dictate more stringent, additional or alternative courses of marrow purging prior to transplantation. More recently Gribben, et al. employed a PCR technique to detect the presence of residual *bcl-2* positive B-cell non-Hodgkin's lymphoma in bone marrow purged *ex vivo* with monoclonal antibody and complement. Post-transplant disease-free survival correlated with completely purged marrow and the inability to deplete residual lymphoma cells was the most important prognostic indicator in predicting relapse. Thus, the ability to detect, at very low frequencies, the presence of leukemic clones is of paramount importance. To address this issue a molecular diagnostic methodology was developed to identify individual B-cell ALL clonotypes with regard to unique immunoglobulin heavy chain variable/diversity/joining gene rearrangements (Ig-HVDJ). Gene amplification of the Big-HVDJ complex was achieved by a negative strand J generic primer composed of Big-HJ2, 3, 6 primers and a degenerate Big-HJ1/4/5 primer, and a complementary positive strand primer derived from each of the Big-HV family-specific genes spanning the 5' HV regions. By sequencing through the VDJ region clonotype-specific primers were developed and used in a secondary PCR to detect, with extreme sensitivity and specificity, unique B-cell clonal repertoires. The strategy is ideally suited for screening bone marrow since it does not require pre-selection to a given antigenic specificity nor, because genomic DNA is utilized as substrate, artificial *in vitro* cell population expansion prior to testing. This approach successfully identified the malignant clones from patients with active ALL and provided for the detection of minimal residual disease in autologous bone marrow.

T 302 ALTERATIONS OF THE BCL-2, C-MYC AND P53 GENES IN DIFFUSE LARGE CELL LYMPHOMAS.

Michèle Farrugia, Li-Juan Duan and Neil Berinstein, Departments of Medicine and Immunology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.

The development of diffuse large cell lymphomas is thought to be dependent upon multiple genetic events. In many cases, large cell lymphomas arise from pre-existing t(14;18) positive follicular lymphomas, sometimes exhibiting an additional translocation involving the C-MYC gene. Other cases of large cell lymphoma lack the t(14;18). Recently it has been established that alterations of the P53 tumour suppressor gene occur late in the progression of several different types of malignancies. In our attempt to understand oncogene co-operation in the generation of large cell lymphomas, we assessed the BCL-2, C-MYC and P53 genes in large cell lymphoma cell lines and primary tumour biopsies

In eight large cell lymphoma cell lines studied, we identified three cell lines with the t(14;18) and p53 abnormalities. The p53 abnormalities were 1) p53 mutation accompanied by p53 allelic loss, 2) two p53 mutations and 3) very low expression of p53 mRNA. Two of these cell lines also had rearrangements of the C-MYC gene. In addition, two t(14;18) negative cell lines had p53 mutations acompanied by allelic loss, one of which also had a rearranged C-MYC gene.

Analysis of fresh tumour samples identified two of eleven large cell lymphomas with both the t(14;18) and a p53 mutation and allelic loss, but no C-MYC rearrangements. No p53 mutations were identified in ten follicular lymphoma samples.

Our data suggests that events that contribute to the development of some large cell lymphomas may involve co-operation between mutant p53 genes, BCL-2 and C-MYC overexpression.

T 303 **HETEROGENEITY OF CLONAL T-CELL RECEPTOR BETA CHAIN VARIABLE REPERTOIRES IN ADULT T-CELL LEUKEMIA, Steven J. Greenberg, Teri F. Beers, Youngnim Choi, Michael H. Rickert, Phyllis M. Overturf, and Tian-Long Du, Department of Neurology, Roswell Park Cancer Institute, Buffalo, NY 14263.**

The molecular immunologic profile of T-cell receptor beta chain (TCR-ß) variable gene (V) usage in adult T-cell leukemia (ATL) was determined. In this disease, the human T-cell leukemia virus type I (HTLV-I) is a necessary but not sufficient step leading to leukemogenesis. The role that TCR-ßV repertoire selection may play in transformation is not known, but restricted heterogeneity might result from recruitment by an anti-retroviral immune response, i.e. mono-family-specific or oligo-family-specific (superantigen) response. Alternatively, the TCR-ß promoter region contains an NF_K-ß binding motif that is potentially inducible via the retroviral transactivating element and unequal activation could result in preferential expression of certain TCR-ßV repertoires. To evaluate the TCR-ßV repertoire, a rapid and sensitive PCR strategy that utilizes genomic DNA was used to screen peripheral blood mononuclear cells from nine patients with active ATL. In all but one case, the clonal TCR-ßV repertoire was identified. In one case, a pattern of biallelic TCR-ßVDJ clonal rearrangement was suggested. The pattern of distribution of TCR-ßV usage was not restricted among the 24 known and characterized ßV families and suggested that VDJ combinatorial events during T-cell maturation and retroviral antigen stimulation or transactivation are not primal events related to the leukemic process in ATL.

T 304 SUBCELLULAR LOCALIZATION OF BCL-2: THE HYDROPHOBIC CARBOXYL TERMINAL DOMAIN TARGETS BCL-2 TO MEMBRANES, Brian Leber, Fabiola Janiak and David W. Andrews, Departments of Biochemistry and Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada L8N 3Z5

The cellular function of the product of the bcl-2 proto-oncogene is to inhibit programmed cell death (*apoptosis*) in systems with a high cell turnover rate. The mechanism by which Bcl-2 affects apoptosis is unknown. Determining the precise intracellular location of the protein is a critical step in elucidating this mechanism. Although previous work has suggested that Bcl-2 is membrane associated, the exact subcellular target membrane is still controversial. We have investigated the membrane association of Bcl-2 in a cell-free system. Bcl-2 was incubated post-translationally with either microsomal membranes, rat heart mitochondria, the P-100 fraction of Rat-1 cells, or artificial phospholipid vesicles. In all cases Bcl-2 associated with the membranes: KOAC was not able to inhibit this association, indicating that it is not due to non-specific electrostatic interactions. Identical results were obtained with cytochrome b5, a protein known to insert post-translationally into membranes. Constructs were made encoding a fusion protein containing the IgG binding domains of S.aureus Protein A, primate globin and the hydrophobic tail of Bcl-2. The Globin/Pr-A passenger protein does not, by itself, associate with membranes. Similar to Bcl-2, the fusion protein with the Bcl-2 tail associated post-translationally with microsomal membranes and intact mitochondria. Furthermore, this protein was not able to be extracted with carbonate, indicating the bcl-2 tail is inserted into these membranes. However, this association was exclusively to the cytoplasmic side of the organellar membrane. These results suggest that the hydrophobic tail of Bcl-2 targets the protein to the outer, not the inner mitochondrial membrane, with the bulk of the molecule exposed to the cytoplasm.

T 305 P.VABEC: A NEW CHEMOTHERAPY REGIMEN FOR AGGRESSIVE NON HODGKIN'S LYMPHOMAS (NHL) IN ELDERLY PATIENTS, Martelli M., Guglielmi C., Amadori S., Romani C., Giovannini M. , Bizzoni L, Mandelli F., Hematology, Human Biopathology Dept. Univ. "La Sapienza"Via Benevento 6 ,Rome 00161 Italy.

A new weekly chemotherapy regimen denominated P-VABEC was employed, from October 1988 to December 1990, in 60 previously untreated elderly patients (pts) aged >60 yrs with aggressive NHL. P-VABEC includes the alternated somministration of: Adriamycin (30mg/sqm), Etoposide (100mg/sqm), Cyclophosphamide (350mg/sqm), at weeks 1-3-5-7-9-11; Vincristine (1,2mg/sqm), Bleomycin (5mg/sqm) at weeks 2-4-6-8-10-12. Oral Prednisone (50mg) was given daily during the entire treatment. Median age was 67 yrs (60-80). Histologic types included 59 diffuse large cells and 1 small non cleaved NHL. Nineteen pts had a stage II, 25 III, and 16 IV. High level LDH was present in 27 (45%) pts and Bulky disease in 7 (12%) pts. Response was evaluated and treatment completed after 8 (first 26 pts) or 12 (following 34 pts) weekly cycles. Fourty-five (75%) achieved a CR, 10 (17%) a PR, no response 5 (8%). So far 21 pts have relapsed (18 CR, 3 PR) and 3 pts died while in CR. After a median follow-up of 25 months actuarial 2 yrs overall survival (OS), DFS and EFS were respectively 64%, 57% and 55%. No statistical difference for OS, DFS, and EFS were observed between the group treated with 8 or 12 cycles. Hematological toxicity was mild in all patients, however a worst neurological and cardiovascular toxicity was observed in pts treated with 12 cycles compared to those treated with 8 cycles. Only one toxic death from lung fibrosis was observed.P-VABEC chemotherapy is an active and tolerable first line chemotherapy in aggressive NHL in elderly pts. Randomized studies are needed to establish the real advantage of this regimen as compared to standard chemotherapy.

T 306 QUANTITATION OF FOLLICULAR NON-HODGKIN'S LYMPHOMA CELLS CARRYING T(14;18) BY COMPETITIVE POLYMERASE CHAIN REACTION, Jules P.P. Meijerink, Toon F.C.M. Smetsers, John M.M. Raemaekers, Theo de Witte, Ewald J.B.M. Mensink, Division of Haematology, Department of Medicine, University Hospital St. Radboud Nijmegen, Geert Grooteplein zuid 8, 6500 HB, the Netherlands.

A competitive Polymerase Chain Reaction (PCR) technique was developed to quantify residual malignant cells in the peripheral blood and bone marrow of patients with low-grade follicular non-Hodgkin's lymphoma (NHL) carrying a t(14;18) translocation. Artificial segments were constructed imitating a translocation between chromosome 14 and 18. These artificial translocation segments were used as competitor molecules in the quantitative PCR. Serial dilutions of patient-derived translocation segments were coamplified with a fixed number of competitor molecules, and a patient specific calibration curve was constructed. Several patient samples were coamplified with an equal number of competitor molecules and the number of t(14;18) translocations within the samples was calculated by comparison with the calibration curve. Using this method, we determined the number of malignant cells in samples of four follicular NHL patients. In a patient transplanted with allogeneic bone marrow declining numbers of residual lymphoma cells were observed.

T 307 INVESTIGATION OF THE PATTERN OF EXPRESSION OF HUMAN TRITHORAX-LIKE GENE H*trx*1 IN HUMAN TISSUES AND LYMPHOCYTES. Pauline Parry*, Malek Djabali*, Jason Khristich*, Marian Waterman=, Mark Bower+ and Glen A Evans*. *The Human Genome Centre and =DBL, The Salk Institute For Biological Studies, La Jolla, CA 92138, USA. +ICRF Medical Oncology Unit, St Bartholomews Hospital, London, UK.

The chromosomal region 11q23 is involved in a number of translocations associated with acute lymphocytic leukemia and acute myeloid leukemia. The site of the breakpoint in the cell line RS4;11 has been cloned and characterized. Analysis of a series of leukemic patients with t(9;11) and t(4;11) translocations indicates that the breakpoints occur in one intron within a 5kb cluster. The cDNA corresponding to the region around the breakpoints has been cloned and characterized. We have named this gene human trithorax-like (H*trx*1) due to its homology to the *trithorax* gene (*trx*)of Drosophila which is particularly significant within the zinc finger domains of the *trx* protein. In drosophila *trx* positively regulates the homeotic genes of the bithorax and antennapedia complexes. In mammals homeobox- containing genes have been shown to be expressed in haemopoetic cells. It is proposed that the H*trx*1 gene acts as a regulator of transcription factors necessary for early haemopoetic development and that disruption of the gene may lead to cells with the leukemic phenotype.

Using part of the H*trx* 1 cDNA we have identified 3 main transcripts which are differentially expressed. Transcripts of approximately 16, 13 and 11kb are recognized in T lymphocytes. In pre-B and B lymphocytes only the 16kb transcript is detected. This pattern of expression, together with the fact that in drosophila *trx* is a transcription factor and that the gene is associated with the leukemic phenotype, is an indication that H*trx*1 may be important in establishing and/or maintaining haemopoetic differentiation. In adult tissues transcripts are detected primarily in brain, smooth muscle and pancreas, with a much lower level of accumulated message in other tissues. Expression of the gene from t(4;11), t(6;11) and t(X;11) chromosomes is also being investigated.

T 308 OVEREXPRESSION OF A TRUNCATED MYB ONCOPROTEIN INDUCES *IN VIVO* B CELL LYMPHOMAS.
Richard D. Press[1], E. Premkumar Reddy[2], and Donald L. Ewert[3]. University of Pennsylvania, Dept of Pathology and Laboratory Medicine[1], The Fels Cancer Research Institute[2], and The Wistar Institute of Anatomy and Biology[3], Philadelphia, PA 19104.

The c-myb proto-oncogene encodes a nuclear DNA-binding phosphoprotein with sequence-specific transcriptional transactivation activity. Structurally modified forms of the myb gene have been found in a variety of naturally-occurring hematopoietic tumor cell types, each of which encodes a protein with deletions of coding sequence at either or both ends of the polypeptide relative to full-length c-myb. To directly assess the role of these terminal deletions in the activation of the *in vivo* oncogenic ability of myb, we have produced retroviruses carrying amino- or carboxyl-terminal truncated versions of myb by introducing these truncated myb genes into a replication-competent avian retroviral vector (RCAMV)*. These myb viruses were injected intravenously into chicken embryos, and the resulting chicks were followed for the presence of myb-induced tumors.

At the age of approximately 5-7 weeks, approximately 20-25% of the birds infected with the virus encoding either an amino- or carboxyl-terminal truncated myb were found to have diffuse involvement of their livers, spleens, kidneys, and bone marrows with an aggressive tumor having a histologic phenotype consistent with a lymphoma. The lymphoma cells expressed cell surface antigens (including IgM) of the bursal B cell lineage consistent with this tumor being a B cell lymphoma. The lymphoma cells expressed the appropriately-sized truncated myb protein within their nuclei and contained integrated myb proviruses of the predicted size. None of the control animals infected with the RCAMV virus without a myb insert developed a similar disease, suggesting a direct lymphomagenic activity for myb proteins with losses of amino- or carboxyl-terminal sequences.

*Press, R.P., A. Kim, D.L. Ewert, and E.P. Reddy. 1992. Transformation of chicken myelomonocytic cells by a retrovirus expressing the v-myb oncogene from the long terminal repeats of avian myeloblastosis virus but not Rous sarcoma virus. J. Virology **66**:5373-5383.

T 309 HOX11, A HOMEOBOX GENE DEREGULATED BY THE t(10;14) of HUMAN T CELL ALL IS NORMALLY EXPRESSED IN THE HINDBRAIN OF THE DEVELOPING EMBRYO.
Charles W. M. Roberts, Masahiko Hatano, Takumi Kawabe and Stanley J. Korsmeyer, Howard Hughes Medical Institute, Washington University School of Medicine, St.Louis,MO 63110.

Molecular cloning of the recurrent t(10;14)(q24;q11) breakpoint of T cell acute lymphoblastic leukemia demonstrated a 2.1 kb transcript that is markedly overexpressed in leukemic T cells when compared to normal thymocytes or several T cell lines. Characterization of this gene from chromosome segment 10q24 revealed it to be a new 330 amino acid homeobox gene, Hox11.

A survey of normal adult tissues by northern analysis revealed little expression of Hox11. Since many homeobox genes are expressed during embryogenesis, *in-situ* hybridization was used to detect expression of Hox11 in the developing mouse. To date, Hox11 transcripts have been detected in the developing branchial arches and hindbrain of day E8.5 through E14.5 embryos. The positive signal has first been detected at E8.5-9.0 in the branchial arches. Upon expression in the mesenchyme of branchial arch 1, induction of Hox11 expression occurs in the overlying dermal tissue. Over the next few days of development, Hox11 is expressed in the migrating neural crest tissue of cranial nerves V, VII and potentially VIII, and IX as well. In the hindbrain, Hox11 expression has been detected from E10.5 to E14.5 in discretely localized areas consistent with expression in the motor nuclei of cranial nerves V, VII, and possibly IX. We postulate that Hox11 may be important in implementing a program of gene expression important for certain aspects of craniofacial development. Hox11 is the first homeobox gene implicated in T cell neoplasia. These studies provide insight into the normal function of Hox11 and will aid the characterization of its role in oncogenesis.

T 310 LENTIVIRAL INTEGRATION IN HIV ASSOCIATED T-CELL LYMPHOMAS,
Bruce Shiramizu, Brian Herndier, and Michael McGrath, University of California, San Francisco General Hospital, Departments of Pediatrics, Pathology and Laboratory Medicine, AIDS Program, San Francisco, CA 94110

Over the past three years, approximately 5% of lymphomas diagnosed and immunophenotyped among HIV-infected individuals at San Francisco General Hospital were of T-cell origin. Two recent cases have been analyzed in detail after Southern blot analysis of the tumor DNA demonstrated that both cases showed evidence for clonally integrated HIV. The first index case consisted of large pleomorphic cells expressing HIV p24 antigen and IL-2 receptor (CD25) and was negative for B-cell, monocyte, and myeloid markers. Both tumors contained a single clonally integrated HIV-1 and were negative for HTLV-I and EBV. To further extend the analysis of the lymphomas, inverse PCR (IPCR) was used to map the integration sites of the two cases. Using primers within the LTR regions, amplified fragments were obtained which consisted of the LTR region flanked by genomic sequences. Direct sequencing of the PCR products demonstrated that both T-cell lymphoma specimens had an LTR region integrated within the exon X of the human *fur* gene. The *fur* gene is located just 5' to the *c-fes/fps* proto-oncogene on chromosome 15. Although both tumors had integrated viral sequences in the same region, the location of the integration sites differed between the two by over 1kb. In order to verify the integration within the *fur* region, Southern analysis was performed, which showed co-migration of bands using *fur* primers and one of the amplification primers, which contains the LTR segment. *C-fes/fps* transcripts from the tumors showed the expected message size of 3kb, which appeared to be upregulated when compared to an uninvolved lymphoid tissue of one of the cases. These cases involving HIV-integration upstream to a known oncogene, *c-fes/fps,* suggest that HIV may be directly involved in tumorigenesis in this particular subclass of lymphomas in HIV-infected individuals.

T 311 INACTIVATION OF THE *Bmi-1* ONCOGENE IN MICE RESULTS IN SEVERE HEMATOPOIETIC DEFECTS AND NEUROLOGICAL ABNORMALITY,
Nathalie M.T. van der Lugt, Koert Linders, Jos Domen, Marian van Roon, Els Robanus, Hein te Riele, Maarten van Lohuizen,Martin van der Valk and Anton Berns, Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

The *Bmi-1* oncogene was first identified as a common proviral integration site in Moloney Murine Leukemia virus induced B-cell lymphomas in Eμ-myc transgenic mice (van Lohuizen et al., Cell 65: 737-752, 1991). The gene is expressed in all tissues, but the highest levels are observed in thymus, spleen, heart, testis and brain. The gene encodes a highly conserved nuclear protein of 324 amino acids, which contains structural motifs present in transcription factors. A murine homolog of *Bmi-1* (*Mel-18*) has been isolated from a melanoma cell line (Tagawa et al., J. Biol. Chem. 265: 20021-20026, 1990). The *Mel-18* gene is expressed in brain and testis only. We decided to inactivate the *Bmi-1* gene in the germline of mice via homologous recombination in embryonic stem cells, to gain more insight in the function of the gene.

Mice heterozygous for the inactivated *Bmi-1* allele are viable, healthy and fertile. From heterozygote X heterozygote crosses, the expected number of homozygous null-mutant mice are obtained. However, in the first days after birth, a significant part of these homozygous mice die (probably caused by underdevelopment of the lungs).

Homozygous mice that do survive are twice as small as their control littermates and develop neurological abnormalities like an instable uncoordinated gait and seizures. The most dramatic defect however is the severely decreased number of T- and B-lymphocytes and myeloid cells in bone marrow, thymus and spleen, concomitant with the reduced size of the latter two organs and bone marrow infiltrated by fat-cells. The remaining cells show a severely impaired response to M-CSF, IL7, IL7 + Steel Factor and LPS in bone marrow colony-assays and an impaired proliferative response of splenocytes to LPS and ConA.

Further developments in the analysis of the phenotype of these *Bmi-1* knock-out mice will be presented.

T 312 **CONSERVED TRANSCRIPTIONAL REGULATORY ELEMENTS OF THE HUMAN AND MURINE BLR1 GENE CODING FOR A NOVEL CYTOKINE RECEPTOR EXPRESSED IN B CELL NEOPLASIA,** Wolf I., Emrich T., Kaiser E. and Lipp M., Institut für Biochemie der Ludwig-Maximilians-Universität, Am Klopferspitz 18a, D-8033 Martinsried, FRG

Comparison of Burkitt lymphoma (BL) cells with immortalized lymphoblastoid B cells by subtractive hybridization led to the identification of the complementary DNA clone BLR1 (Burkitt's lymphoma receptor 1) which encodes a novel member of the GTP-binding (G) protein-coupled receptors. BLR1 mRNA is expressed in Burkitt's lymphomas and lymphatic tissues but not in any other cell types tested. This exclusive expression of BLR1 and the oncogenic potential of this receptor class supports the hypothesis that BLR1 may have a regulatory function in BL lymphomagenesis and/or B cell differentiation. The protein sequence of 372 amino acids is highly related to that of receptors for the cytokine interleukin-8 (IL-8) and other neutrophil chemoattractants.
In the search for distinct factors responsible for the differentiation-specific control of BLR1 expression we have cloned the promoter region and mapped the transcriptional start site using primer extension analysis. Within the upstream promoter region we have identified putative binding-sites for NFκB (-269, -132, +43), c-*ets*-1/PU.1 (-207) and LEF (-393). All these binding sites for transcriptional activators are also highly conserved in the promoter of the murine homologue of BLR1. Cotransfections with expression plasmids of the NFκB subunits p50 and p65 result in a significant increase of the transcriptional promoter activity measured by CAT-assay. Analysis of promoter deletion constructs will be used to elucidate the role and interaction of factors necessary for positive or negative regulation of the BLR1-gene expression during B-cell differentiation.
We conclude that BLR1 may represent a potential candidate involved in the process of physiologic trafficking, cell-cell interaction, and activation of mature B lymphocytes in lymphatic tissues.

Cell Biology/Lymphokines

T 400 IMMUNOTHERAPY WITH RECOMBINANT IL2 (rIL2) IN THE TREATMENT OF MALIGNANT LYMPHOMAS (L), D.Blaise, C.Gisselbrecht, N.Vey, JL Pico, P.Tiberghien, N.Milpied, B.Coiffier, M.Attal, A.Bosly, J.Reiffers, H.Tilly, A.Tabilio, R.Gabus, M.Brandely and D.Maraninchi, Institut Paoli Calmettes and Inserm U119, Marseille, France.

Previous studies have reported some response with rIL2 in ML. In order to evaluate the place of IL2 in this setting we treated 80 pts with ML in two situations. 55 pts with progressive disease under chemotherapy (low grade M2:n=22 ; high grade ML (n=22) ; Hodgkin disease (n=7) ; mycosis fungoides (MF) (n=4)) received rIL2 (provided by Roussel Uclaf) as *continuous infusion*. 20 Millions IV/m2 was administrated over 3 cycles (C1:5 days ; C2:4Days ; C3:3 Days) on D1, 15 and 29. Treatment toxicity leaded to stop treatment in 11 pts. Complete and partial response was achieved in 10 pts (low grade:1/22 ; high grade:5/22 ; MF:4/4).
25 pts (high grade=14;Hodgkin disease=11) received rIL2 (12 to 24 M IV/m2 as a continuous infusion over 5 cycles on day 1 (C1=5 days) 15, 29, 43 and 57 (C2 to 5=2 days eachs) in an average of 58 days after auto BMT prepared with BEAC regimen. At time of transplant, 3 pts were in first CR (HG=2 ; HD=1) ; 9 pts were in CR or sensitive relapse (HG=7 ; HD=2) and 13 pts were refractory (HG=5;HD=8) overal toxicity was manageable and related to upper dose levels treatment was associated with a high immune stimulation. With an average follow up of 24 mths DFS for HG and HD is 63% and 51%. Comparison with historical control groups is favorable in non refractory HG ML and invite further studies.

T 401 **HIGH LEVELS OF CIRCULATING SOLUBLE RECEPTORS FOR TUMOR NECROSIS FACTOR IN HAIRY CELL LEUKEMIA AND TYP B CHRONIC LYMPHOCYTIC LEUKEMIA,**
Digel W, Lindemann A, and Mertelsmann R. Department of Internal Medicine I, University of Freiburg,D-7800 Freiburg, FRG

Tumor necrosis factor (TNF) is a pleiotropic cytokine that plays a major role in inflammation,and host defence to infection; it has also been shown that TNF stimulates leukemia growth in patients with hairy cell leukemia (HCL), type B chronic lymphocytic leukemia(B-CLL), and acute myelgenous leukemia.We have investigated the presence of soluble tumor necrosis binding proteins (TNFBP) in the sera of healthy volunteer blood donors and cancer patients.Two distinct types of TNFBP, Type A and B, which are immunologically related to the cellular 75-kD TNFR and the cellular 55-kD TNFR, respektively,were assessed by immunoassays using nonblocking anti-receptor antibodies and 125I-rhTNF alpha.As compared to the titers observed in 25 healthy controls,TNFBP types A and B titers were found to be elevated in almost all sera obtained from patients with underlying malignant disease.The highest amount of TNFBP were seen in the sera of patients with B cell malignancies including HCL and B-CLL. The most notable result was the excepionally high TNFBP with predominance of type A over type B TNFBP in the sera of most HCL and B-CLL patients.Effective treatment of HCL patients with rhIFN alpha was associated with decrease of circulating TNFBP.Analysis of the cellular source of TNFBP indicated,that the neoplastic B-cells are the producer cells of TNFBP.TNF serves as an autocrine growth factor in both disease states and thus TNFBP may play an important role in the regulation of neoplastic cell growth.

T 402 EVALUATION OF IMMUNOTOXINS FOR CLINICAL APPLICATION IN HODGKIN'S DISEASE, Andreas Engert, Volker Diehl and Philip Thorpe Universitätsklinik I, Köln, Germany; University of Texas, Texas, USA.

Hodgkin's disease is an ideal candidate for immunotoxin therapy since the putative malignant Hodgkin/ Reed-Sternberg (H-RS) cells express high amounts of target antigens like CD25 and CD30 which are present only on a small minority of normal cells. We evaluated more than 60 monoclonal antibodies for potential use as ricin A-chain immunotoxins against H-RS cells. Immunotoxins were constructed by linking the antibody moiety via a sterically hindered linker to deglycosylated ricin-A (dgA). Selection procedures included screening for crossreactivity on normal human tissue sections, toxicity tests against Hodgkin cells in vitro, treatment of nudemice with solid Hodgkin tumors, and SCID mice with disseminated human Hodgkin tumors. The most potent immunotoxin, RFT5.dgA (CD25), was as effective as whole ricin against H-RS cells and bound strongly and specifically to H-RS cells in all Hodgkin's tissue sections. In addition, RFT5.dgA destroyed more than 60% of solid H-RS tumors of 0.5 cm diameter in nudemice and induced 80% lasting complete remissions in SCID mice with disseminated Hodgkin's lymphoma. RFT5.dgA is currently being evaluated in clinical phase-I trials in patients with refractory Hodgkin's disease.

T 403 CARE: HIGH DOSE CYTOSINE ARABINOSIDE AND ETOPOSIDE IN RELAPSED AND REFRACTORY NON-HODGKIN'S LYMPHOMA. Gibson J, Mansberg R, Joshua DE and Kronenberg H. Haematology Department, Royal Prince Alfred Hospital, Missenden Road, CAMPERDOWN NSW 2050 AUSTRALIA.

With conventional anthracycline based therapy the outlook for patients with relapsed or refractory NHL is poor with less than 10% responding to further treatment. An alternative approach (for patients able to tolerate therapy) involves the use of high dose chemotherapy followed by autologous bone marrow transplantation (ABMT). We have evaluated a high-dose regimen employing cytosine arabinoside and etoposide (CARE) in such patients prior to ABMT. The protocol involves low dose Ara-c (50mg/m^2 over 12 hours) over the nights of days 1-5. On days 2-5 the patient also receives Ara-c 3g/m^2 and etoposide 200mg/m^2 each over one hour. Intrathecal treatment is given as indicated. The study group consisted of 12 patients with immediate or high grade NHL who had initially received anthracyline containing therapy. Five had initially responded and one had achieved a PR. The remaining six were considered to have been primarily refractory. All patients were treated with one or two cycles of CARE and assessed for response. Six patients achieved a CR and remain in remission from 5-48 months from the date of chemotherapy. One patient achieved a PR and five have had no response. The overall response rate was thus 58%. All CR patients have subsequently undergone successful autologous transplants using a combination of marrow and PBSC and remain in remission from 1 to 44 months. The nadir granulocyte count was <0.1x10^9/l in 75% and 11 patients had 13 episodes of neutropenia requiring hospital admission and parenteral antibiotics. The median time to absolute neutrophil count of 0.5x10^9/l was 19 days. The median nadir platelet count was 9x10^9/l and the median time to a platelet count greater than 50x10^9/l was 17 days. By comparison non-haematological toxicity was mild. Grade three mucositis was seen in one patient whilst another suffered from prolonged diarrhoea. CARE thus yields results comparable with other salvage regimens and the response duration in patients achieving CR has been durable and encouraging, permitting successful ABMT. Non-haematological toxicity has been mild whilst growth factors may have a role in ameliorating haematological toxicity.

T 404 DIFFERENTIAL EXPRESSION OF CYTOKINES IN HUMAN LYMPHOMAS AND LEUKEMIA, Kube D.[1], Fanai S.[1], Straub H.[1], Ludwig W.D.[2], Tesch H.[1], Diehl V.[1], 1-Universität Köln, I. Medizinische Klinik, 5000 Köln 41, 2-Freie Universität Berlin, Universitätsklinikum Steglitz, 1000 Berlin 45.

Cytokines may regulate the growth and differentiation of normal hematopoietic cells and are possibly involved in the biology of malignant lymphoma and leukemia.
To analyze the role of cytokines in human lymphoid neoplasia, we have studied as a first step the transcription of interleukins in acute lymphoblastic leukemias, Burkitt's lymphoma and Hodgkin's disease derived cell lines using the polymerase chain reaction. The results are summerized in the table:

	IL3	IL4	IL5	IL7	IL8	IL10
c-ALL	5/12	5/12	0/19	10/19	17/19	17/19
preB/B-ALL	0/6	2/6	0/6	3/13	11/13	12/13
preT/T-ALL	0/6	1/5	0/6	0/13	11/13	2/13
Burkitt's lymphoma	1/18	4/18	4/18	2/18		12/18
Hodgkin' disease	2/8	1/8	2/8	3/8	1/8	4/8
periph. blood lymph.	-	-	-	+	+	+

Thus these data demonstrate that certain interleukins are differentially transcribed in lymphoid neoplasia.

T 405 DIFFERENTIATION-SPECIFIC EXPRESSION OF THE G PROTEIN-COUPLED CYTOKINE RECEPTOR BLR1 IN B CELL DEVELOPMENT AND B CELL NEOPLASIA, Martin Lipp, Edelgard Kaiser, Reinhold Förster, Ingrid Wolf, and Thomas Emrich, Institut für Biochemie der Universität, Am Klopferspitz 18a, D-8033 Martinsried, FRG

BLR1 (Burkitt's lymphoma receptor 1) originally identified by subtractive hybridization from Burkitt's lymphoma represents the first lymphocyte-specific member of the superfamily of G protein-coupled receptors (Eur. J. Immunol. 22: 2795-2799, 1992). We have also isolated the gene encoding the mouse homologue of *BLR1*. It consists of two exons encoding a protein of 374 amino acid residues which shows 83% identity to the human receptor. Sequence comparison revealed a significant relation of both proteins to receptors for the cytokine interleukin-8 (IL-8) and other neutrophil chemoattractants.

In situ hybridization and Northern blot screening of normal tissues of BALB/c mice and primary lymphomas representing different stages of the B cell lineage revealed that murine *blr1*-specific RNA is detected consistently at low levels only in secondary lymphatic organs. Expression is regularly and strongly observed in lymphomas of mature B cells but not in plasmacytomas.

SCID mice, which are deficient in the developement of a mature B cell compartment, exhibited a strongly reduced level of *blr*-1-specific RNA in the spleen. Induction of terminal differentiation of resting B cells towards Ig-secreting plasma cells by cytokines completely downregulated expression of *blr*-1. This remarkably restricted expression pattern of *blr*-1 as well as its relation to chemokine receptors suggests that BLR1 and its murine homologue may represent a novel cytokine receptor involved in the process of migration, cell-cell contact and activation of mature B lymphocytes.

T 406 LYMPHOKINE GENE EXPRESSION AND EFFECTS ON CELL PROLIFERATION IN AIDS-ASSOCIATED NHL.
Michael S. McGrath, Jane Marsh, Timothy Nolan and Brian Herndier, Departments of Laboratory Medicine, Medicine and Pathology, AIDS Program, University of California, San Francisco and San Francisco General Hospital, San Francisco, CA 94110

AIDS associated non-Hodgkin's lymphomas represent a broad spectrum of molecular and immunophenotypic entities. In order to test whether lymphomostimulatory cytokines might play a role in lymphomagenesis we analyzed 16 primary AIDS lymphoma specimens and 4 AIDS NHL cell lines for the expression of cytokine genes by RT-PCR. All specimens were negative for IL-1, IL-2, IL-3, IL-4, IL-5, and IL-7 gene expression. 14/16 tumors and 4/4 cell lines expressed IL-10; 13/16 tumors and 2/4 cell lines expressed IL-6 receptor (IL-6R); and 4/16 tumors and 2/4 cell lines expressed IL-6. The 2 IL-6 and IL-6R expressing cell lines were outgrowths of tumors initially presenting as peritoneal effusions containing cells morphologically and immunologically consistent with large cell lymphomas. *In vitro* growth control studies demonstrated IL-6 dependent proliferation in one case where associated fibroblasts were found to be the source of IL-6. The other case where IL-6 was produced by the tumor cells was growth inhibited in a dose dependent manor by IL-4. Because IL-6 levels are markedly increased in HIV-infected individuals tumors found to only be expressing IL-6R may also have their growth influenced by IL-6. These studies show that AIDS associated NHL's frequently express IL-10 and IL-6R and that IL-6 may serve as a paracrine or autocrine growth factor not unlike what has been observed for human multiple myeloma.

T 407 INTERACTIONS OF ANTI-B4-BR WITH CHEMOTHERAPEUTIC AGENTS ON B-ALL CELLS *IN VITRO*.
Rosemary O'Connor, Victor S. Goldmacher, Beverly A. Teicher, Walter A. Blättler, ImmunoGen, Inc., 148 Sidney St., Cambridge, MA 02139 and Dana-Farber Cancer Institute, 44 Binney St., Boston, MA 02115.

Combination chemotherapy has significantly contributed to the achievement of complete remissions in B cell acute lymphoblastic leukemia (B-ALL) and lymphoma. However, many patients eventually relapse, and most relapses are attributed to the survival of drug resistant tumor cells. One potentially useful strategy for eliminating more of the residual resistant cells is to combine conventional chemotherapeutic drugs with immunotoxins. Anti-B4-bR is an immunotoxin consisting of the anti-CD19 monoclonal antibody anti-B4, conjugated to ricin in which the galactose binding sites of the B chain have been chemically blocked (bR) to eliminate non-specific binding. Compared with alkylating agents or metabolic inhibitors, immunotoxins have different mechanisms of cellular uptake and cytotoxic action (ricin kills cells by catalytic inactivation of ribosomes), hence such combinations should have non-overlapping modes of resistance.

We investigated the cytotoxic effects *in vitro* of combinations of anti-B4-bR with adriamycin, 4-hydroperoxy-cyclophosphamide (4HC), etoposide and cisplatin on the following cell lines: Namalwa (a Burkitt's lymphoma derived B cell line); Namalwa/mdr-1 (Namalwa cells expressing the multiple drug resistance protein); and the ALL-3 cell line (derived from a pre-B ALL). Cytotoxicity assays were performed by treating the cells with anti-B4-bR and a drug simultaneously for 24 or 72 hours, or by pre-exposing cells to anti-B4-bR for 24 hours followed by exposure to drug for 1 hour. The data were subjected to isobologram analysis. Combinations of anti-B4-bR with adriamycin or cisplatin appeared to produce additive to supra-additive killing of each cell line under most conditions. There was additive killing when 4HC and anti-B4-bR were combined. This enhanced or additive cytotoxic effect on cells *in vitro* of several immunotoxin/drug combinations warrants further investigation in pre-clinical studies.

T 408 ENDOTHELIAL ADHESION MOLECULES IN NORMAL AND MALIGNANT LYMPH NODES
Timo Paavonen and Risto Renkonen, Departments of Pathology, and Bacteriology & Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland.

Several endothelial adhesion molecules mediate leukocyte extravasation through high endothelial venules (HEV). The integrins ICAM-1 and -2 and VCAM-1 can mediate lymphocyte, monocyte and granulocyte adhesion to endothelial cells (EC), their counter-receptors being CD11a/18&CD11b/18 and CD11a/18 and VLA-4, respectively. In vitro many cytokines can increase the expression of ICAM-1, VCAM-1, but not the expression of ICAM-2 on endothelial cells. In this study we compared the reactivity of monoclonal antibodies (Mab), directed against endothelial adhesion molecules ICAM-1 (clone 84H10), ICAM-2 (6D5), and VCAM-1 (4B9) in hyperplastic and malignant lymph nodes. Frozen sections and standard immunoperoxidase staining methods were used. The reactivity with ICAM-1, ICAM-2 and VCAM-1 Mab is stronger in HEV and other smaller vessels in lymphomas compared with hyperplastic nodes. There was practically no ICAM-2 staining in non-malignant lymph nodes. VCAM-1 Mab reacted also strongly with germinal centers.

The leukocyte traffic into lymph nodes is also regulated by the expression of selectin family (L-, E-, and P-selectin) on leukocytes and their ligands on HEVs. E- and P-selectins recognize sialyl-Lewis x and -Lewis a carbohydrate motifs, and we have shown elsewhere that this is the case also with L-selectin (ref.1). However there was no difference on the level of endothelial (capillaries and HEVs) expression of these motifs (Mabs: anti-sLex; CSLEX and anti-sLea; Ca 19-9) on benign or malignant lymph nodes.

Using Mab 6D5 we have shown that ICAM-2 expression is upregulated on endothelial cells in lymphoid malignancies. This might have important implications for the regulation of leukocyte traffic and inflammation. No differences were seen on the expression of selectin family counter receptors, ie. sialyl-Lewis x and -Lewis a carbohydrate motifs.

Ref. 1. Paavonen, T., and Renkonen, R. Am. J. Pathol. 141, 000-000, 1992, in press.

T 409 TRANSDUCTION OF HETEROLOGOUS CYTOKINE/RECEPTOR GENES WITH COMMON PROMOTER MOTIFS DISTINGUISHES LEUKEMOGENESIS IN HTLV-I INFECTION.
Christopher Palma, Jean Chen, Edward Klein, Kazuo Fujihara, and Steven J. Greenberg, Department of Neurology, Roswell Park Cancer Institute, Buffalo, NY 14263.

The disparate up-regulation of certain genes in adult T-cell leukemia (ATL) may reflect an event at the molecular level related to leukemogenesis. Infection with the human T-cell leukemia virus type I (HTLV-I) is an initial and necessary, but not sufficient event that leads to the development of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and ATL. A second, perhaps unrelated and independent event during the course of HTLV-I infection results in the development of the leukemic state in ATL. There are a number of genes whose expression is upregulated in ATL, but not in HAM/TSP or sero-positive carriers (SPC), and this observation may reflect genetic transduction related to the transforming mechanisms active in the leukemia. To further explore this hypothesis, we compared the expression of interleukin-2 receptor alpha chain (IL-2Rα), transforming growth factor-β$_1$ (TGF-β$_1$) and intracellular adhesion molecule-1 (ICAM-1) in ATL, HAM/TSP and SPC. IL-2Rα, TGF-β$_1$ and ICAM-1 levels of transcription were determined by quantitative RT-PCR, and serum protein levels by ELISA. Controls included mycosis fungoides/Sezary, multiple sclerosis, and normal individuals and productive and defective HTLV-I-infected T-cell lines. Vastly increased levels of mRNA transcription and protein for IL-2Rα and TGF-β$_1$ were detected in ATL as previously reported. In addition, ICAM-1 was discovered to be greatly upregulated in ATL compared to the other HTLV-I-infected and non-infected states. ICAM-1 has not been previously recognized to be selectively induced and expressed along with IL-2Rα and TGF-β$_1$ in ATL and not in HAM/TSP or SPC. Collectively, these three genes exhibit unrelated coding regions but share common promoter motifs that may shed insight into the process and maintenance of the leukemic state.

T 410 RADIOLABELED ANTIBODY THERAPY OF B CELL LYMPHOMAS, Oliver W. Press, Janet F. Eary, Christopher C. Badger, Paul J. Martin, Frederick R. Appelbaum, Wil B. Nelp, Darrell Fisher, Dana Matthews, and Irwin D. Bernstein. Departments of Medicine, Nuclear Medicine, and Pediatrics, University of Washington, Seattle, WA 98195, The Fred Hutchinson Cancer Research Center, Seattle, WA 98104, and The Batelle Pacific Northwest Laboratories, Richland, WA 99352.

We have evaluated the biodistribution and therapeutic potential of I-131-labeled anti-B cell monoclonal antibodies (MoAbs) in 44 patients (pt) with advanced non-Hodgkin's lymphomas who failed conventional chemotherapy and/or radiation therapy. Sequential MoAb biodistribution studies were performed on successive weeks with escalating amounts of antibody (0.5, 2.5, 10 mg/kg) trace-labeled with 5-10 mCi I-131. Absorbed radiation doses to tumor sites and normal organs were estimated by the Mirdose method based on data obtained by serial whole body gamma camera imaging, serial tumor biopsies, and computed tomography. In all cases, larger antibody doses (e.g. 2.5 or 10 mg/kg) yielded better antibody biodistributions than lower MoAb doses (0.5 mg/kg). In 24 of the 44 patients, every assessable tumor site received more radiation than any critical normal organ, and these patients were considered candidates for therapeutic infusions of I-131-MoAbs. The 20 pt who did not achieve favorable MoAb biodistributions generally had large tumor burdens (>0.5 kg, 13 pt) or massive splenomegaly (14 pt). Three of the 24 pt with favorable MoAb biodistributions developed human anti-mouse antibodies before radioimmunotherapy could be undertaken, and 2 pt were unable to be treated for logistic reasons. The other 19 "favorable" pt were hospitalized for 6-8 days and given 59-1168 mg of anti-B cell antibodies (anti-CD37 MoAb MB-1, 6 pt; anti-CD20 MoAb 1F5, 1 pt; anti-CD20 MoAb B1, 11 pt; anti-idiotypic antibody,1 pt) labeled with 232-777 mCi of I-131. Acute toxicity was limited to transient nausea, although all treated patients experienced significant myelosuppression 2-4 weeks following treatment. Fifteen pt had elective reinfusion of autologous, purged bone marrow. Fifteen of the pt achieved complete remissions and 8 remain in continuous complete remission after 4-50+ months. Two pt achieved a partial response, one pt a minor response, and one pt is too early to evaluate. We conclude that the tolerable toxicity and encouraging efficacy warrant further dose escalation in this phase 1 trial. (Supported by NIH Grant CA 44991).

T 411 THE ROLE OF rhGM-CSF IN THE CONVENTIONAL POLYCHEMOTHERAPY OF HIGH GRADE NON-HODGKIN'S LYMPHOMA, Pier Luigi Zinzani, Sante Tura, Giuseppe Papa, Renato Fanin, Massimo F. Martelli, Piero Galieni, Giuseppe Leone, Teodoro Chisesi, Vincenzo Liso, Franco Dammacco, Bruno Rotoli, Luciano Moretti, Antonio Cuneo, Ettore Volpe, Marco Gobbi, Aurelio Cajozzo, Federico Calabresi and Franco Mandelli, Italian Cooperative Study Group on Malignant Lymphoma, Institute of Hematology, Bologna University, 40138 Bologna, Italy

In a prospective multicenter randomized study we are evaluating the F-MACHOP regimen in high grade non-Hodgkin's lymphomas at the diagnosis. The criteria of eligibility are the following: histology according to G, H, and J categories of the Working Formulation; stage II-IV; age less than 60 years; HIV negativity. On the basis of the good CR rate and FFR obtained with this polichemotherapeutic regimen in our previous multicenter randomized trial testing F-MACHOP versus MACOP-B and in order to examine the role of dose intensity on treatment outcome and the potential role of rhGM-CSF to decrease morbidity and to allow repeated cycles of relatively safe dose-intensive treatment, we proposed to test the F-MACHOP regimen randomizing the patients in two arms: rhGM-CSF versus no rhGM-CSF. A minimum of four F-MACHOP cycles are given to each patient; upon the completion of four cycles, patients will be restaged: all patients in a complete remission or in "significant" partial remission, will continue induction therapy with other 2 F-MACHOP cycles. The arm rhGM-CSF treatment consisting of administration of rhGM-CSF 5 μg/kg/day injected SC from day 8 (4 days after the regimen end) of each treatment cycle to day 18.

The objectives are: i) to calculate the dose intensity of drugs actually delivered with F-MACHOP; ii) to evaluate the role of rhGM-CSF in decreasing morbidity, in obtaining a higher neutrophil nadir, in repeating cycles of relatively safe dose-intensive treatment. So far, 177 patients entered in this study and in the next months we will have the preliminary data.

Late Abstract

EPSTEIN-BARR VIRUS (EBV) RELATED LYMPHO-PROLIFERATION WITH SUBSEQUENT EBV NEGATIVE T LYMPHOMA-DETECTION OF EBV LATENT MEMBRANE PROTEIN (LMP) IN CD3+, CD4+ AND CD8+ T CELLS, Q.Tao, G.Srivastava, F.C.S.Ho, S.L.Loke, R.Liang* and Y.T.Liu. Department of Pathology and Medicine*, University of Hong Kong, Hong Kong.

Presence of EBV genome and expression of EBV encoded proteins have been shown in a significant portion of T or B cell lymphomas which suggested that EBV may have a role in the pathogenesis of some lymphomas. In the present case, the patient developed generalized lymphadenopathy with spontaneous regression after lymph node biopsy. Histology showed disturbed architecture with preponderance of large B -blasts mixed with numerous CD8+ T-lymphocytes, consistent with an acute EBV infection. Immunohistology and gene rearrangement studies confirmed the absence of clonal lymphoproliferation. Polyclonal EBV genome was detected in the nodal tissue by Southern blot analysis. Expression of EBV proteins (EBNA2, LMP, ZEBRA) was detected in a proportion of cells and EBV lytic proteins (EA, VCA, MA) were also detected in rare scattered cells by immunostaining method. Double immunostaining combining APAAP and indirect fluorescence showed LMP expression in less than 10% of T and B cells and the LMP+ cells consisted of 26% CD2+, 23% CD3+, 8% CD4+, 17% CD8+, 73% CD19+ cells. Macrophages were exclusively negative for LMP. Lymphadenopathy recurred eight months later and biopsy showed a T cell lymphoma with clonal T cell receptor beta-chain rearrangement. However, EBV genome was not detectable in DNA extracted from the tumour tissue by Southern blot analysis, and also the immunostaining for EBNA2, LMP and ZEBRA was negative. This case is interesting in that T cell lymphoma occurred after an episode of EBV related reactive lymphoproliferation and the selected T cell for clonal expansion may not necessary be the EBV containing cell. The possibility of EBV acting as a co-factor in such events would be presented.