GASTRIC MALT LYMPHOMA: FROM CONCEPT TO CURE
Peter G Isaacson. Department of Histopathology, Royal Free and University College Medical School, London WC1E 6JH

Gastric MALT lymphomas are clinically and histologically quite distinct from comparable low-grade B-cell lymphomas of lymph nodes. Their histology suggests that immunological mechanisms might be operative in their growth. Given that there is normally no lymphoid tissue in gastric mucosa and that *H. pylori*, the only common bacterial antigen in the stomach, results in the accumulation of gastric MALT, the possibility that this organism is implicated in the pathogenesis of gastric lymphoma has been extensively investigated. It appears that most, but not necessarily all, gastric MALT lymphomas arise in MALT acquired in response to *H. pylori* infection and develop by stepwise accumulation of genetic abnormalities. Early molecular events in the evolution of gastric MALT lymphoma from “acquired” MALT include trisomy 3, t(11;18)(q21;q21), genetic damage leading to genetic instability, as indicated by the so-called replication error repair (RER) phenotype, and both p53 and c-myc mutations. At this stage in their development, the growth of the lymphomas is driven by contact between the neoplastic B-cells and *H. pylori* specific intra-tumoral T-cells. Eradication of *H. pylori* causes the tumour to enter a latent phase resulting in clinical regression. Later events, such as t(1;14)(p22;q32), appear to be linked to a capacity for autonomous growth, loss of sensitivity to *H. pylori* and dissemination of the lymphoma beyond the stomach and gastric lymph nodes. Cloning of the breakpoint in t(1;14) has allowed the identification of a new tumour suppressor gene, bcl10. High grade transformation of MALT lymphoma has been associated with p53 inactivation, deletions of p16 and t(8;14).
Oral Presentations

1. Virology

EPSTEIN-BARR VIRUS AND LYMPHOMAGENESIS
A.B. Richardson
CRC Institute for Cancer Studies, University of Birmingham, Birmingham, B15 2TT, U.K.

Epstein-Barr virus (EBV) is a human gamma herpesvirus with cell growth transforming ability which efficiently colonises the B lymphoid system. The growth transforming infection in essence is controlled by cytotoxic T lymphocyte (CTL) surveillance directed against virus latent cycle antigens. Virus persistence depends upon the establishment of a pool of non-cycling memory B cells which carry the virus genome but express only a limited number of viral antigens.

Though the virus carrier state is usually asymptomatic, in rare circumstances it can give rise to three distinct types of EBV-positive B cell lymphoma, each with a different pathogenesis and with a different range of viral proteins expressed. These are:

(i) post-transplant lymphoproliferative disease which at least in its initial stages is directly EBV-driven, expresses the full spectrum of latent proteins and remains susceptible to a restoration of CTL surveillance.
(ii) Burkitt's lymphoma, a tumour of germinational centre origin where virus antigen expression is restricted to EBNA1 and where defects in antigen processing function allow efficient tumour cell escape from CTL detection.
(iii) Hodgkin's Disease, a post-germinal centre tumour, where expression of the latent membrane proteins LMP1 and 2 (in addition to EBNA1) renders the malignant cells potentially immunogenic to the CTL response. The prospects for successful immunotherapy of EBV-positive Hodgkin's Disease are discussed in terms of the available epitopes, their ability to be presented by Reed-Sternberg cells and the possibility that responses are suppressed in the vicinity of the tumour.

PRESENCE OF HHV-8 IN THE BONE MARROW AND PERIPHERAL BLOOD DENDRITIC CELLS OF PATIENTS WITH MULTIPLE MYELOMA
R. Vescio, H. Ma, N. Sjäck-Shieh, L. Zhang, G. Chen, M. Podol, G. Schiller, S. Gao, T. Moos, J. Said, J. Bermanoff, West LA VAMC and UCLA, Los Angeles, USA; University of Texas @ San Antonio, San Antonio, USA; BIS Laboratories, Los Angeles, USA.

Introduction: HHV-8 is a gamma herpesvirus which contains human homologues to genes such as IL-6 and IL-8 that could potentially promote multiple myeloma (MM) cell growth. We have previously reported the existence of HHV-8 genome within cultured bone marrow (BM) dendritic cells and fresh BM biopsies in MM patients using PCR and in situ hybridization, respectively. Additional experiments were performed to clarify the role HHV-8 may have in MM pathogenesis since conflicting results have subsequently been published both corroborating and refuting these initial findings.

Methods: BM and peripheral blood samples were obtained from normal individuals and patients with MM or MGUS. Peripheral blood dendritic cells (PBDCs) were collected by enrichment for PBMCs expressing CD80 and CD83 with an immunomagnetic bead column. BM dendritic cells were cultured from aspirate material as previously described.

Results: Using primers specific for ORF26, HHV-8 was detectable in only 3% of the PBDCs derived from normal patients vs. 35% and 64% of PBDCs obtained from MGUS and MM patients, respectively. Since the virus may be sexually transmitted, we searched for HHV-8 in the close contacts of patients with MM. HHV-8 was detected in only one of the latter 46 samples (2%, p<0.001). Representative expression analysis was performed to identify genes overexpressed in MM BM dendritic cells. In both sets of experiments, the HHV-8 VIF homolog was isolated. Expression of this gene was then confirmed using RT-PCR in BM biopsies derived from 14 of 17 MM patients. Western blot analysis confirmed vIRF protein production in all 18 MM (PCR+) samples tested. HHV-8 ORF74 (vIRF-R) transcripts were also identified using RT-PCR in these biologies (18 of 22 MM samples positive). None of the cultures derived from normal bone marrow showed expression of either gene. ORF26 (n=25) and ORF65 (n=14) were sequenced in patients with detectable virus and -but only rare intra-patient differences were noted. Most (92%), of the MM patients were infected with strain A. A consistent deletion at position 11227 near the 3' end of ORF26 was seen in all patients.

Conclusions: Our results provide further evidence that HHV-8 exists in the majority of patients affected with MM. Lack of serological evidence of infection and negative virus PCR results by some groups can likely be explained by differences in the viral strains in these patients.

EPSTEIN-BARR VIRUS USURPS TUMOR NECROSIS FACTOR AND NOTCH RECEPTOR PATHWAYS TO ALTER CELL GROWTH
K. Izumi, G. Mosiaoulos, E. MacFarland, J. Lin, B. Sylva, O. Devergne, K. Kaye, M. Higuchi, A. Cooper and E. Kieff
Channing Laboratory, Harvard 181 Longwood, Ave, Boston, MA, 02115

To establish long term latency in human B lymphocytes, EBV expresses 6 nuclear proteins (EBNAs) and 2 integral membrane proteins (LMPs) which cause lymphocyte proliferation that can be malignant. EBNA-2, -3A, -3B, and -3C associate with RBP, the principal nuclear effector of Notch signaling, while EBNA-LP markedly up-regulates the activity of the EBNA-2 acidic domain in transcriptionally activating cellular and the viral LMP promoters that have RBP binding sites. The EBNAs also engender immune T cell response in vivo. The immune T cells contain the growth of EBV infected cells and can be expanded, in vitro, to prevent or treat EBV induced lymphoproliferations (Rooney et al; O'Reilly et al). EBV infection is also associated with the development of B and T cell lymphomas, Hodgkin's disease, anaplastic nasopharyngeal carcinoma (NPC) and some gastric carcinomas. Only EBNA1, LMP1 and LMP2 are characteristically expressed in the late onset malignancies.

LMP1 has profound effects on EBV infected cell growth and survival. LMP1 functions as a constitutively activated receptor because its six hydrophilic transmembrane domains cause ligand independent aggregation in the plasma membrane. The C-terminal cytoplasmic domain has two sites that are critical for transmitting cell growth and survival signals. Site 1, near the plasma membrane, is essential for transformation, engages TRAFs, activates NF-kB and AP1, and upregulates expression of TRAF1, EB13, and EGFR. Site 2, at the C-terminus, enables efficient long term outgrowth, engages TRADD and other DD proteins, and activates NF-kB and AP1. Despite engagement of DD proteins Site 2 does not mediate pro-apoptotic effects. The 120 residues between Site 1 and 2 are dispensable for lymphocyte growth transformation.

EPSTEIN-BARR VIRUS PROVIDES PROLIFERATIVE ADVANTAGE TO REED-STERNBERG CELLS IN CLASSICAL HODGKIN'S DISEASE AND SURVIVAL ADVANTAGE TO THE PATIENT
Department of Pathology, Tata Memorial Hospital, Mumbai, India and Lymphoma Biology Section, National Cancer Institute, Bethesda, USA.

Introduction: Most Epstein-Barr virus (EBV) associated lymphoproliferative disorders have high proliferation indices. However, classical Hodgkin's disease (CHD) is heterogeneous, both with respect to the proliferation index of the reactive neoplastic cell and the Reed-Sternberg cell (RS cell), and with respect to EBV-association. Hence, we investigated whether the proliferation index of the RS cells differs in CHD with and without EBV-association. We also investigated the relevance of EBV-association with respect to survival.

Material & Methods: We investigated 110 cases of CHD for association with EBV and for proliferation in RS cells. EBV-association was identified by immunohistochemical demonstration of EBV-latent membrane protein-1 expression and by demonstration of EBV encoded nuclear RNA 1 (EBER1) by mRNA in situ hybridization. Proliferation was assessed by immunostaining for antibody to proliferating cell nuclear antigen (PCNA). Relevant univariate and multivariate tests were carried out.

Results: EBV-association was noted in 86 of 110 cases (78.2%). Higher PCNA expression, age <15 years and mixed cellular histology correlated independently with EBV-association. Sixty-two of 66 cases (93.9%) with PCNA expression in >50% of RS cells were EBV associated while 24 of 44 cases (54.5%) with PCNA expression in <50% of RS cells were associated. EBV-association was noted in 49 of 50 patients (98%) in the age group of <15 years and 27 of 65 (41.5%) in the age group of >14 years showed EBV-association. EBV-association was noted in 86.5% of mixed cellularity and in 68.3% of nodular sclerosis subtypes. A longer overall survival was seen in patients associated with CHD. Patients with PCNA expression in >50% of RS cells and those in stages I & II had a higher relapse free survival.

Conclusion: Our study suggests that EBV is likely to confer a higher proliferative potential to RS cells in CHD. The EBV-association or the associated change in the proliferative status of the RS cells may be responsible for longer survival in this group of patients.

1. Virology
CD81, a receptor for Hepatitis C Virus is a component of a B Cell Signalling complex: Shohara Levy1, Mike Pinto2, Catherine Maskell2, Larry D. Leoniis-Prieto1, Christine Shotton2, Jean Duboys2, Peter Monti3, Adrian Higginbottom2, and June A. McEntee2. Stanford University1; Reading University1; Henry M. Jackson Foundation1; Institut Pasteur de Lille1; University of Sheffield1.

CD81 (TAPA-1) is a widely expressed tetraspanin molecule involved in an astonishing variety of biologic responses and has been cloned repetitively. Cloning of CD81 was achieved independently by the use of mAbs that induced a variety of biological effects; including changes in cell adhesion, morphology, activation, proliferation and differentiation of lymphoid and neuronal cells. Most recently CD81 was identified as the ligand for Hepatitis C Virus (HCV) E2 glycoprotein (Pileri et al. Sciences 282:938, 1998).

We produced a soluble truncated version of the HCV envelope glycoprotein E2. Interaction of E2 with cells was dependent upon the expression of human or chimpanzee CD81. The E2 protein did not bind cells expressing African Green Monkey (AGM) CD81, which differs from that of human or chimpanzee CD81 at only four amino residues. Binding of E2 to a human CD81 expressing B cell line induced cellular adhesion and an anti-proliferative effect, similar to that induced by the anti-CD81 mAbs, suggesting that CD81-dependent signalling events may be required for HCV infection and replication. To further study CD81-E2 interactions we produced a soluble recombinant form of human CD81 (GST-CD81) and have shown that it binds E2. A similar construct derived from AGM CD81 did not bind E2. Moreover, GST-CD81 inhibits the binding of E2 to CD81 expressing cells. Mutagenesis studies defining the contact sites of interactions between HCV and its receptor will be presented.

On B cells CD81 is a component of a molecular complex which includes the complement receptor CR21, the B cell specific protein, CD19, and the interferon inducible protein, Leu13/9-27. This molecular complex reduces the threshold for antigen-specific B cell activation via the B cell receptor by bridging Ag specific recognition and CD21-mediated complement recognition. It is of note that the receptor for the Epstein-Barr Virus (EBV) is CD21. Thus HCV may predispose B cells to malignant transformation by binding to the CD81 molecule thereby enhancing signal transduction through the B cell receptor.
BIOLOGY OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS
D. Liebowitz, Leonard and Madlyn Abramson Family Cancer Research Institute of the University of Pennsylvania Cancer Center, Department of Medicine, Hematology-Oncology Division, University of Pennsylvania, Philadelphia, PA, USA

Post-transplant lymphoproliferative disease (PTLD) is recognized as a heterogeneous group of lymphoproliferations occurring in patients immunosuppressed after solid organ or T cell-depleted bone marrow transplantation. The majority of these cases are of B cell origin and are Epstein-Barr virus (EBV)-associated. PTLD ranges from a pre-malignant polyclonal expansion of EBV-infected B cells to monoclonal aggressive NHL. In order to understand the pathogenesis of PTLD, the ability of EBV to stimulate the proliferation of infected B lymphocytes, the role of the host immune system in controlling EBV-induced B cell proliferation and molecular alterations in the proliferating B cells as malignant clonal evolution occurs, must all be considered. EBV encodes an array of latent viral gene products. The pattern of EBV gene expression in PTLD will be discussed. The role of one EBV protein, the latent membrane protein 1 (LMP1) in the evolution and maintenance of PTLD will be described in detail. The molecular and cytogenetic changes that occur in PTLD will be described, in the context of how they contribute to the evolution of the disease. Based on the data presented a model for the pathogenesis of PTLD will be presented.

DEVELOPING STRATEGIES FOR ADOPTIVE CELL THERAPY OF TRANSPLANT RELATED EBV ASSOCIATED LYMPHOMA.
R. O'Reilly, New York, USA

ABSTRACT NOT RECEIVED

Post-Transplant Lymphoproliferative Disorders: Diagnosis and Treatment. Lode J. Swinnen, M.D., Loyola University Chicago.

Immuno-suppression-related B-cell disorders are seen after organ transplantation and in congenital and acquired immuno-suppression states. Post-transplant lymphoproliferative disorders (PTLD) comprise a histologic spectrum ranging from hyperplastic appearing lesions to frank non-Hodgkin's lymphoma or multiple myeloma histology. Multiple clones may co-exist, representing a uniquely different mechanism for lymphomagenesis. The incidence varies from 1% in renal recipients to 8% in lung recipients, but can be markedly increased by the use of anti-T cell therapies, or by T cell depletion in bone marrow transplantation. Pre-transplant EBV seronegativity increases risk to as high as 30-50%. >50% of tumors are EBV-associated. Mechanisms for viral lymphomagenesis remain incompletely defined; LMP-1 may function as an oncogene and coprecipitates with TAMP, bcl-2 overexpression has also been identified. A possible direct tumorigenic effect has recently been suggested for cyclosporine. PTLD has a highly variable clinical picture, certain patterns are however seen. Reversalability of PTLD with reduction in immunosuppressive has long been recognized. Predicting reversibility has been difficult. The presence or absence of bcl-6 mutations has recently been identified as being of predictive value. Surgical resection can be curative. Cytotoxics, although problematic, can also be curative. Long term remission has been achieved with anti CD20 and CD24 antibodies; efficacy has been reported anecdotally for interferon alpha and for rituximab. In vitro expanded EBV-specific T cells have been effective as treatment and as prophylaxis in the setting of bone marrow transplantation. EBV viral load measured in blood appears to correlate with the emergence of PTLD and may facilitate prophylactic studies. PTLD is a model of immuno-suppression related EBV lymphomagenesis. Pathogenetic, therapeutic, and prophylactic insights gained from the study of PTLD are likely to be applicable to the AIDS setting.

CHROMOSOMAL ABNORMALITIES IN POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLD) BY COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

Introduction : PTLDs are a heterogeneous group of disorders, mostly developed from B-cell and chromosomal abnormalities are informative to better characterize these tumors.

Methods: To study chromosomal abnormalities, 22 PTLDs were analyzed using CGH and FISH techniques on interphase nuclei. All PTLDs were classified with the morphological and genealogy data to identify the following categories: Polymorphic Polyclonal (PP): 4 cases, Polymorphic Monoclonal (PM): 7 cases, Monomorphous Monoclonal (MM): 9 cases. In this study 1 case of Hodgkin Disease (HD), and 1 case of myeloma were included.

Results: Morphological and molecular data with CGH were compared showing a high incidence of chromosomal abnormalities in PM and MM.

Histological types Number of cases genetic abnormalities - genetic abnormalities +
PP 4 1 1
PM 7 3 4
MM 9 2 7
HD 1 1
Myeloma 1 1

In the 14 cases with chromosome abnormalities, over expression of the long arm of chromosome 8 containing C-MYC was seen in 2 PM, HD and myeloma. In the last one, over expression of the short arm of chromosome 2 containing N-MYC, this abnormality was also present in one case of MM in association with an over representation of the long arm of chromosome 18.

In another case of MM several abnormalities were associated with an amplification of the 1q21-qter segment. For the last case BCL2 amplification was confirmed by FISH on nuclei using yacs of BCL2 region.

Conclusion: This study of PTLDs by CGH has shown the complexity of chromosomal abnormalities in the MM type of PTLDs in correlation with the worse prognosis of this subtype of PTLDs.

2. Lymphoma in Transplanted and Immunodeficient Patients
HUMANIZED ANTI-CD20 MONOClonAL ANTIBODY (RITUXIMAB) IN B POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (B-PTLDs): A RETROSPECTIVE ANALYSIS OF 32 PATIENTS


Introduction: Anti-B cell monoclonal antibodies have been proved to be effective in B PTLDs (Blood. 2002;99:3137). Other treatments such as chemotherapy or immune drugs are toxic or ineffective. We report the activity of Rituximab (Mabthera®, Roche) in 32 B PTLDs treated in 14 French centers.

Patients and methods: There were 8 liver, 8 kidney, 6 bone marrow (BM), 4 heart, 3 lung, 1 heart lung, 1 kidney-pancreas, and 1 liver-pancreas transplant recipients. Median age was 13 years (3-67 years) and the median delay between graft and tumor was 6 months (1-156).

In organ recipients, tumors were classified as polymorphic and monomorphic in 10 and 13 cases respectively (3 unclassified cases). Tumors were associated with EBV in 22 out of 26 tested cases. 4/6 BM recipients were treated without pathological documentation because of a rise of EBV load, fever and lymph nodes enlargement. Immunosuppressive regimen were modified in 27 pts. Rituximab was used as first line therapy in 30 pts and as salvage therapy in 2 pts with 8 infections (2 pts), 4 infections (2 pts), 3 infections (1 pt) and 2 infections (3 pts) of 375 mg/m2.

Results: Tolerance was good. The response was evaluable in 26 pts (treatment or evaluation are ongoing in 6 pts). In organ recipients, 9 pts were in CR, 4 in PR (± 3, 5, 5, 8 months). In BM recipients, 5 were in CR. The response in all pts was 14/26 CR (54%) and 18/26 CR+PR (69%). 6 pts died (2 in CR) and relapse was observed in 2 pts (16 and 9 months). 2 pts are alive, 10 in CR, 4 in PR, in failure and 6 are under evaluation with a median follow-up of 5 months (1-12 months).

Conclusions: The use of Rituximab appears to be a safe and relatively efficient therapy in B PTLDs. The results need to be confirmed in a prospective multicentric trial.

HUMAN IMMUNODEFICIENCY VIRUS-RELATED LYMPHOMA: CORRELATIONS BETWEEN CLINICAL FEATURES AND HISTOLOGICAL SUBTYPES


Introduction: The increasing incidence of non Hodgkin's lymphoma among patients with human immunodeficiency virus (HIV-1) has led to recognize the role of the HIV in the pathogenesis of the lymphomas. The aim of the study was to evaluate the relationship between clinical and histological features in patients with HIV-1.

Methods: 291 patients with aggressive HIV-1 lymphomatis were included in the three consecutive GELA trials between 1988 and 1997, were selected on the basis of availability of clinical data (initial characteristics and outcome). Histological slides were reviewed by the French Study Group of Pathology.

Results: Patients were classified in five classes as follows: classic large cell lymphoma (DLCL) (45%), Burkitt lymphoma (BL) (14%) [classic BL (C-BL) (15%) and atypical BL (A-BL)], so-called Burkitt-like lymphoma (29%), immunoblastic lymphoma (IB) (13%) and atypical lymphoproliferation (2%). Lymphoma features were compared to non-BL types. BL was more frequently associated with skin involvement, oral cavity, rectum, lung, small bowel, and mesenteric and/or cranial nerve, and liver involvement (37% vs 15% in DLCL and 14% in IB, p=001). 21% vs respectively 6% and 10% of p01, 31% vs 23% and 11% of p01, with higher rate of stage IV (73% vs 57% in DLCL and 64% in IB, p<0.001) and tumor size >10 cm (34% vs 22% and 19% respectively of p01). The C-BL and A-BL types were very similar regarding lymphoma features, and so were the DLCL and IB types. Immune status: Mean CD4 + lymphocyte counts was higher in BL than in DLCL and IB types (223 ± 1071 vs 141 and 116 ± 108 in BL and 88 ± 101 in IB, p=001). Compared to other subgroups, IB subtype had the most unfavourable immune features: higher rate of AIDS prior to lymphoma (33% vs 12% in BL and 14% in DLCL, p=001). More patients with CD4 <100 × 100 /L (61% vs 39% in IB and 42% in DLCL, p=001). Immune characteristics of C-BL were better than those of A-BL (mean CD4 respectively 287 ± 107 vs 186 ± 105, p=001) and 10% of C-BL and 15% of A-BL subtypes had similar immune characteristics. The mean delay between the onset of HIV diagnosis and lymphoma occurrence was 28 months (m) in C-BL, 40 m in A-BL, 46 m in DLCL and 48 m in IB. Response rate to chemotherapy was similar but the overall survival was significantly shorter in C-BL (46% m) than in A-BL (55% m, p=001).

Conclusions: BL showed more aggressive features than non-HIV BL. C-BL occurred in patients than non-BL with the best immunological status; IB presented with the most immunodeficient status and the lowest survival rate.


1. EA 2406. CHU et Université de Bordeaux 2,33167 Bordeaux, France.

2. French Study Group of Cutaneous lymphoma. 3: Laboratoire de Virologie, CHU Bordeaux.

Introduction: HIV-infected patients are at high risk to develop systemic lymphoma but cutaneous lymphomas (CL) are rare and therefore not included among the CDC criteria for AIDS staging. Their pathogenesis and current prognostic meaning are not well understood.

Aim of the study: 21 patients were recorded from our files to determine whether HIV-associated lymphomas with cutaneous presentation share features of primary CL arising fortuitously during HIV infection or whether they represent the cutaneous presentation of AIDS-systemic lymphoma.

Results: Bexide rare mycosis fungoides (n=3), which shared typical clinicopathologic lesions, non-epidermotropic large-cell CL (n=18) were predominant. They mostly presented as solitary nodule or tumor without systemic spreading at presentation. Seven of the 8 large T-cell CL had a CD30 phenotype but did not express ALK protein. EBV-EBER transcripts were detected in 2 of them but LMP1 protein was absent. Except for their original presentation, the features of these T-cell CD30+ CL were the same as in immunocompetent patients. The 10 B-cell CL were either immunoblastic or centroblastic lymphomas expressing either CD45+ or CD45R+, respectively. Four B-cell CL expressed CD30, EBER transcripts, LMP1 and p53 protein, contrasting with the phenotype of B-cell CL in immunocompetent hosts. HHV-8 DNA sequences were detected within CL in a single patient with concomitant Kaposi's sarcoma and Castleman disease. Lastly, all CL occurred in patients with a low CD4 count and death was due to immunodeficiency rather than to lymphoma spreading suggesting to avoid aggressive immunosuppressive treatment. These results were compared with original CL in immunocompetent hosts or AIDS-systemic lymphoma.

2. Lymphoma in Transplanted and Immunodeficient Patients
LYMPHOMA CLASSIFICATION: FROM REAL TO WHO
N.L. Harris, E.S. Jaffe, J. Diebold, G. Flandrin, H-K. Muller-Hermelink, J. Vardiman, WHO Lymphoma Committee. Massachusetts General Hospital, Boston, MA, USA; National Cancer Institute, Bethesda, MD, USA; Hotel Dieu, Paris, France; U. Wurzburg, Wurzburg, DE; Hospital Neckar, Paris, FR; U. Chicago, Chicago, IL, USA

Introduction: The "Revised European-American Classification of Lymphoid Neoplasms" (REAL), published in 1994 by the International Lymphoma Study Group (ILSG), is based on the principle that a classification is a list of "real" disease entities, which are defined by a combination of morphology, immunophenotype, genetic features, and clinical features. The relative importance of each of these features varies among diseases, and there is no one "gold standard." In some tumors morphology is paramount, in others it is immunophenotype, a specific genetic abnormality, or clinical features. An international study of 1300 patients, supported by the San Salvador Foundation, subsequently showed that the REAL classification could be used by pathologists, with inter-observer reproducibility better than other classifications (p<5%). Immunophenotyping was helpful in some diagnoses, but not required for many others. New entities not specifically recognized in the Working Formulation accounted for 27% of the cases. Diseases that would have been lumped together as "low grade" or "intermediate/high grade" in the Working Formulation showed marked differences in survival, confirming that they need to be treated as distinct entities.

Since 1995, the European Association of Pathologists (EAFP) and the Society for Hematopathology (SH) have been developing a new World Health Organization (WHO) classification of hematologic malignancies, using an updated REAL classification for lymphomas and applying the principles of the REAL classification to myeloid and histiocytic neoplasms. The classification of myeloid neoplasms will recognize distinct entities defined by a combination of morphology and cytogenetic abnormalities. A Clinical Advisory Committee (CAC) was formed to ensure that the classification will be useful to clinicians. The CAC concluded that clinical groupings of lymphoid neoplasms was neither necessary nor desirable. Patient treatment is determined by the specific type of lymphoma, with the addition of grade within the tumor type, if applicable, and clinical prognostic factors such as the international prognostic index (IPI).

MOLECULAR PATHOGENESIS OF B CELL LYMPHOMA
Riccardo Dalla-Favera
Department of Pathology, Columbia University, New York, NY 10032

The pathogenesis of non-Hodgkin lymphomas (NHL) represents a multistep process involving the clonal accumulation of genetic lesions affecting proto-oncogenes and tumor suppressor genes. The most common mechanism of genetic lesion is represented by chromosomal translocations that alter the pattern of expression of various proto-oncogenes by juxtaposition of heterologous regulatory sequences. Significant progress has been made in identifying the proto-oncogenes associated with various B cell derived NHL. The features of known chromosomal translocations associated with various NHL subtypes will be presented with particular emphasis on the role of the BCL-6 proto-oncogene which is expressed in all GC-derived NHL and is structurally altered in a substantial fraction of cases. In addition, novel findings on the molecular dissection of certain chromosomal abnormalities involving band 1q21, among the most frequent in NHL, will be presented. The diagnostic and therapeutic implications of these findings will be discussed.


THE NEW WHO CLASSIFICATION OF MALIGNANT LYMPHOMAS - CLINICAL IMPLICATIONS
Authors: W. Hiddemann, M.A. Bast, J. Armitage
Department of Medicine, Ludwig-Maximilians-University, Munich, Germany

Based on the extensive efforts of the International Lymphoma Study Group (ILSG) a worldwide accepted classification of malignant lymphomas has recently been established and also accepted by the WHO. This classification has omitted the general grading of lymphomas into different categories, but has rather followed two main criteria:

1. The designation of lymphomas according to the lineage from which the malignant cells derive (B and T-cell lymphomas)
2. The stage of differentiation (precursor cell lymphomas and "peripheral lymphomas")

This new structure faces the clinician with the challenge to recognize specific subtypes and to select the most appropriate measures for diagnosis and treatment. In order to assess the relevance of this approach in comparison to a previously proposed clinical grouping of lymphoma subtypes dividing them into the three major groups of indolent, aggressive and very aggressive lymphomas, the survival curves of 1,093 patients, who entered into the respective pathobiological and clinical analysis of the International Lymphomas Study Group were evaluated. For this purpose the lymphomas were grouped into the three major clinical groups and were subsequently analysed within each group for the individual histologic subtypes. While the survival curves were rather comparable within the indolent lymphomas, substantial differences were particularly recognized for the aggressive lymphomas. In this group the five year survival rates ranged from 78% for anaplastic large cell lymphomas to 14% for the mantle cell lymphomas with intermediate rates of 38% for diffuse large B-cell lymphomas and 68% for follicular lymphomas grade 3. These data strongly emphasize, that a clinical grouping of different lymphoma subtypes appears inadequate and that an appropriate clinical management requires the need to direct the respective measures to the specific pathobiologic subtypes hence strongly supporting the new WHO concept.

CHARACTERIZATION OF THE GENES REARRANGED IN THE t(11;18)(q21;q21) ASSOCIATED WITH MALT LYMPHOMAS

1D and M.R. contributed equally to the study and should both be regarded as first authors.
1Dep of Oncology and Hematology, University Hospital Eppendorf, Hamburg, Germany; 2Center for Human Genes and Plandis Universitair Institute for Biotechnology, Dep of Pathology, University of Leuven, Belgium; Dep of Pathology, University of Salamanca, Spain

B-cell non-Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT) are the commonest subtype of lymphoma arising at extranodal sites, with most cases originating in the gastric mucosa. They develop in a setting of chronic infection or autoimmune disorders and are characterized by distinct clinicopathologic features. The genetic mechanisms underlying the genesis of MALT lymphomas are not known. The t(11;18)(q21;q21) appears to be the key genetic lesion and is found in approximately 50% of cytogenetically abnormal low-grade MALT lymphomas. Using positional cloning, we were able to identify and characterize the genes involved in the t(11;18). As a first step, we applied FISH using YAC clones to narrow the breakpoints on chromosome 11 and 18, respectively. In both cases studied, the breakpoint on chromosome 11q could be mapped at 105 cM within the non-chimeric YACs 921F3 and 906C5. The breakpoint on chromosome 18 was found to be contained in YACs 949B6 and 817C6 at 81 cM. Subsequently, FISH experiments with PAC clones isolated for ST6s D18S587, D18S1055, and D18S1129 contained in YAC 949B6 were performed. Starting from these PACs a walking strategy was applied and two PAC clones spanning the chromosome 18 breakpoint were identified. The breakpoints were further narrowed down by FISH analysis with BambIII fragments subcloned from these PACs. Based on these data, cloning and characterization of the involved genes was performed. These results will be presented in detail.
BCI10 GENE ABNORMALITY IN B-CELL LYMPHOMAS

H. Peng1, M-Q Du1, T.C. Dias1, A. Poyraz1, A. Dogan1, R. Homoudi2, T. Willis3, L. Pan1, M.J.S. Dyer2, P.G. Isaacson1.

1Department of histopathology, UCL Medical School, London, UK;
2Academic Haematology and Cytogenetics, Institute of Cancer Research, Sutton, UK.

Introduction: Bcl10 is a novel apoptosis regulating molecule recently identified through cloning of the breakpoint of t(1;14)(p22;q32) from a low grade gastric MALT lymphoma. Preliminary transformation study indicates that Bcl10 may behave like a tumour suppressor. To understand its role in lymphomagenesis, we investigated Bcl10 gene rearrangement and mutation in a range of B-cell lymphomas.

Methods: Sixty six cases of B-cell lymphomas of various sub-types were examined for Bcl10 gene rearrangement by Southern blot analysis. These together with an additional 69 cases were screened for Bcl10 mutations by PCR-SSCP analysis and direct sequencing.

Results: Southern blot analysis demonstrated Bcl10 gene rearrangement in only 3 cases which were previously shown to carry t(1;14)(p22;q32) by karyotyping. Abnormal SSCP migration patterns were observed in 70/135 cases. Direct sequencing showed that 21 cases (21/135=15.6%) harboured missense or frame-shift mutations, the remaining cases being either polymorphisms or silent mutations. The majority of these mutations were distributed in the C-terminal of the gene. Multiple mutations were found in 3 cases. Examination of tumour samples from different sites of the same lymphoma in 5 cases showed both common and novel mutations suggesting that mutation may be an ongoing event.

Conclusions: Our preliminary results indicate that Bcl10 was frequently mutated in B-cell lymphoma and Bcl10 mutation was independent of translocation.
4. Biology II

MOLECULAR CHARACTERIZATION OF 11q DELETIONS POINTS TO A PATHOGENIC ROLE OF THE ATM GENE IN MANTLE CELL LYMPHOMA

S. Stiilgenbauer, D. Winkler, G. Ott, C. Schaffner, E. Leupolt, M. Bentz, P. Müller, H.-K. Müller-Hermelink, M.R. James, P. Lichter, H. Döhner. Med. Klinik und Poliklinik V, University of Heidelberg, Germany; Deutsches Krebsforschungszentrum, Heidelberg, Germany; Department of Pathology, University of Würzburg, Germany; Department of Pathology, University of Ulm, Germany; Welcome Trust Centre for Human Genetics, Oxford, England.

Introduction: Deletions involving the long arm of chromosome 11 (11q) were recently shown to be recurrent chromosome aberrations in mantle cell lymphoma (MCL). Neither the minimal deletion region nor candidate tumor suppressor genes involved have been identified.

Methods: In the current study we searched for 11q deletions in 116 MCL by fluorescence in situ hybridization (FISH). The molecular extent of the deletions was determined with a contiguous set of physically mapped yeast artificial chromosome (YAC) probes spanning bands 11q14 to 11q24.

Results: Deletion of chromosome 11 material was observed in 37 of the 116 MCL (32%). The minimally deleted segment lost in all cases analyzed comprised YAC 801e1 containing the ATM gene. To further narrow the minimal region of loss PI-derived artificial chromosome (PAC) probes mapping to the deleted region were isolated and used as probes in cases without aberrations detectable with YAC probes. This allowed the identification of an ATM deletion which was beyond the resolution level of YAC probes.

Conclusions: The identification of a minimally deleted segment specifically affecting the ATM gene together with preliminary data from mutation analyses suggest a pathogenic role of ATM as a tumor suppressor gene in MCL.

DYSREGULATION OF CYCLIN DEPENDENT KINASE 6 (CDK6) EXPRESSION IN SPLENIC LYMPHOMAS THROUGH SPECIFIC CHROMOSOME 7q TRANSLOCATION


Introduction: Splenic lymphomas with villous lymphocytes (SLV) is a chronic B cell lymphoproliferative disorder with characteristic lymphocyte morphology which may represent the leukemic phase of some cases of splenic marginal zone lymphoma (SMZL). We have previously found structural abnormalities of chromosome 7q in 20% of cases of SLV.

Methods: We have investigated three cases of SLV with a 7q22(q71)del(q22) and one case of SMZL with a 7q21(q22)del(q22).

Results: Fluorescent in situ hybridization using a YAC probe assigned to 7q22 showed a split signal in each case. The three patients with SLV gave a split signal with a control probe containing the promotor region and first exon of the CDK6 gene. A split signal was found in the case of SMZL using a control located 50KB distal to the first exon. Using genomic probes derived from both controls rearrangements were found in DNA from tumour cells in all four cases but not from other cases of SLV and SMZL lacking the 7q translocation.

In each case the translocation breakpoint was cloned. In the three cases of SLV, an immunoglobulin heavy variable region gene was juxtaposed to a 1.65 KB region 3.6 KB upstream of CDK6. The 7q breakpoints all showed identity to the immunoglobulin recombinination recognition sequence suggesting that translocations arose as a result of aberrant V(D)J recombination. In the patient with SMZL, the breakpoint was situated 600KB upstream of the SLV breakpoint and appears to join the promotor region of a previously unidentified expressed transcript to this site. In two cases of SLV in which additional material was available. Western blotting and flow cytometry demonstrated overexpression of CDK6.

Conclusions: Overexpression of cyclin D1 and allelic loss of RB1 frequent abnormalities in SLV. We propose that overexpression of CDK6 is a novel additional mechanism which dysregulates the G1 phase of the cell cycle and contributes to the pathogenesis of some cases of SLV and possibly to other B cell lymphoproliferative disorders.

ANTISENSE OLIGONUCLEOTIDES DIRECTED TO THE BCL-2 GENE MESSAGE (G3139) WITH LOW DOSE CYCLOPHOSHAMIDE CURE ESTABLISHED SYSTEMIC HUMAN B CELL LYMPHOMA IN SCID MICE

R. Klasa, M.P. Wong, R.D. Gascoune, M.B. Bally

Departments of Advanced Therapeutics and Pathology.

British Columbia Cancer Agency, Vancouver, BC, Canada.

Introduction: Antisense oligonucleotides (ASO's) directed at bcl-2 have been shown to specifically downregulate message and protein expression with resultant therapeutic effect in models of lymphoma. An 18-mer ASO directed at the first 6 codons of the human bcl-2 message (G3139, Genta Inc) was combined with cyclophoshamide (CPA) in an attempt to enhance the therapeutic potential.

Methods: SCID Rag-2 mice were intravenously injected with 5x10^6 (t14;18) bearing, bcl-2 overexpressing, DoHH-2 human lymphoma cells. Cohorts of 6 mice began treatment with: CPA 35 mg/kg intraperitoneal (IP) on day 4, 8 and 12; G3139 5 mg/kg IP alternate days for 28 days (14 treatments); combined CPA with G3139; CPA with reverse polarity sense bcl-2 ASO's; CPA with 2-base mismatch bcl-2 ASO's; the two control ASO's alone.

Results: Median survival for untreated and ASO control animals was 33 days. Animals treated with CPA alone or CPA with control ASO's survived 47 days. G3139 alone resulted in 60 day median survival with 30% long-term survivors. Bcl-2 ASO's with CPA cured 80% of animals (n=30 mice) with no histological evidence of tumor at sacrifice on day 100.

Conclusion: ASO's directed at bcl-2 (G3139) in combination with a chemotherapeutic agent show clinical synergy in this pre-clinical model. A phase I/II human trial is currently underway based on these findings.

BCL-2 INSERTION INTO THE IG H LOCUS IN FOLLICULAR LYMPHOMA


Leiden University Medical Center, Leiden, The Netherlands.

Using interphase and fiber FISH we have previously analyzed a series of follicular lymphomas for BCL-2 gene rearrangements. In 440 cases the BCL-2 gene was found to have two translocation breakpoints on the same allele; one 3' and one 3' of the gene. In two cases, fiber FISH showed 'juxtaposition' of the IgH locus to the 3' breakpoint, and the D and V genes to the 3' breakpoint. Southern blotting with an nbo probe showed no rearrangement with commonly used enzymes, suggesting that the breakpoints were located several kb away from the nbo. Southern blotting with a BCL-2 exon2 probe and a probe upstream of the gene showed that the 5' breakpoints were located between these probes. By inverse PCR with primers upstream of the BCL-2 gene, the fusion products of the BCL-2 and 3' flanking regions were cloned.

Sequence analysis showed that both 5' breakpoints were 57 bp apart, and located within a CA-repeat ±600 bp upstream of the BCL-2 gene. The two 5' breakpoints were only 2 bp apart and located ±7 kb 5' of the nbo. Sequencing of 1 kb around the 3' breakpoints region revealed that the breakpoints were within a charlik2 repeat element and within a cluster of 4 topoisomerase II (topo II) consensus recognition sequences. Using primers for the JH and DH genes, the fusion products of 5'BCL-2 with JH and 3'BCL-2 with DH were PCR-cloned. Sequence analysis showed that in both cases the 5'BCL-2 breakpoint was juxtaposed to the JH gene. The 5' breakpoint was juxtaposed to D44-23 in one case and to J3-J5 in the other. In all 6 fusion products N nucleotides were inserted. Both in the J and D genes the breakpoints were at heptamer-nonamer signal sequences, implying RAU12-mediated VDJ recombination at the cause of chromosome 14 breakage. At the BCL-2 breakpoint regions, no such signal sequences were present. The fact that the 3' breakpoints were close together but far from the common breakpoint clusters suggests that the mechanism of breakpoint induction in these cases is different from normal (t14;18). The presence of clustered topo II consensus sequences at the 3' breakpoint and of CA repeats, which have been shown to be sensitive to topo II cleavage, at the 5' breakpoint, points to a possible involvement of topo II in chromosome 18 breakpoint induction. Of note, these insertions would be undetectable by karyotyping and the commonly used PCR and Southern blot assays.
RECURRENT IMMUNOGLOBULIN GENE TRANSLOCATIONS IDENTIFY DISTINCT MOLECULAR SUB-TYPES OF MYELOMA

P.L. Bergsagel, M. Chesi, and W.M. Kuehl
New York Presbyterian Hospital - Weill Medical College, New York, NY and National Cancer Institute, Bethesda, MD

Introduction: Chromosome translocations involving the immunoglobulin heavy chain gene (IGH) on 14q32 are a seminal event in the pathogenesis of many B cell malignancies. We hypothesized that since myeloma is a tumor of nature, isotype switched plasma cells, the 14q32 translocations would occur in the switch regions of the IGH locus.

Methods: We analyzed a panel of 21 human myeloma cell lines using a Southern blot assay to detect illegitimate rearrangements involving the switch regions, we then cloned and mapped the breakpoints, developed probes for FISH analysis, cloned and studied the novel oncogenes.

Results: Despite the fact that by karyotypic analysis only half of the cell lines demonstrated a 14q32 abnormality, we found evidence of IGH translocations in every cell line. In five cell lines there is a 1(11;14), with three cloned breakpoints occurring 100-230kb centromeric to cyclin D1. In one cell line there is an insertion of the IGH switch 'circle' excised from the productive allele, into 11q13, with the insertion breakpoints 12kb centromeric to cyclin D1. These six cell lines, and no others, express a high level of cyclin D1. In five cell lines there is a (14;16), with four cloned breakpoints 100-500kb centromeric to c-maf, in one cell line there is a (16;22) with the breakpoint telomeric to c-maf, serving to delimit the region. These six cell lines, and no others, express a high level of c-maf. In five cell lines there is a (6;14) with breakpoints 50-100 kb centromeric to FGF3. These five cell lines, and no others, express a high level of FGF3. These breakpoints fall within the telomeric introns of a novel SET domain protein, MMSET, and are associated with IGH-MEESET hybrid mRNA transcripts. The remaining five cell lines have translocations involving other loci: 8q24, 6p25 (MUM1), 21q22.

Conclusions: Ig translocations identify at least four distinct molecular subtypes of myeloma. 25% 11q13 with c-maf, 25% 16p16 with FGF3 and IGH-MMSET hybrid transcripts, 25% others. The clinical and phenotypic consequences of these subtypes remains to be determined.

Gain of chromosome arm 9p is characteristic of primary mediastinal B-cell lymphomas

Martin Bentz, Thomas F. E. Barth, Dahlia Bock, Alfred C. Feller, Michael J. Schwerter, Stefan Joos, Michael Baudis, Hartmut Döhner, and Peter Müller
1Medizinische Klinik und Poliklinik V, Universität Heidelberg; 2Abteilung Pathologie der Universität Ulm; Pathologisches Institut der Medizinischen Universität Lübeck; 3Abteilung Pathologie des Bundesesikerkrankenhauses Ulm; and 4Deutsches Krebsforschungszentrum, Heidelberg, Germany

Introduction: Primary mediastinal B-cell lymphomas (MBL) is an aggressive, extranodal Non-Hodgkin’s lymphoma (NHL) presenting with anterior mediastinal involvement. Although MBL may be cytologically indistinguishable from large cell lymphomas of other sites, it exhibits distinctive clinicopathologic and immunohistochemical features. In a recent molecular cytogenetic study, a high frequency of gains on chromosome arm 9p was described.

Methods: We investigated 352 B-cell neoplasms, (27 Burkitt’s lymphomas, 78 follicle center lymphomas, 40 mantle cell lymphomas, 60 chronic B-cell leukemias, 72 diffuse large cell lymphomas, 31 primary gastrointestinal large B cell lymphomas and 44 MBL) for the presence of such 9p gains by fluorescence in-situ hybridization (FISH) with a DNA probe mapping to chromosomal band 9p21 or by comparative genomic hybridization.

Results and Conclusions: 30 of 40 MBL (75%) exhibited 9p gains by FISH analysis, and this aberration was present in 21 of 44 MBL analyzed by CGH (48%). In 308 cases of other B-cell neoplasms, only six 9p gains were identified (2%). These data show that gains of chromosome arm 9p are highly characteristic of MBL and support the view that MBL is not only characterized by its specific anatomic localization, but that there are biologic differences between MBL and other indolent and aggressive B-cell NHLs. Moreover, FISH using a DNA probe mapping to 9p could become a useful diagnostic procedure in addition to the histopathological evaluation of mediastinal lymphomas.

A cell epitope determined with random peptide libraries and combinatorial peptide chemistry stimulates T cells specific for cutaneous T cell lymphoma

Thomas Linnemann, Keld Kalloft, Kari-Heniz Wesmüller, Walther Syrry and Peter Walden
Department of Dermatology, Medical Faculty Charité, Humboldt University, Berlin, Germany

Introduction: Mycosis fungoides is the most frequent T cell lymphoma of the skin. Despite numerous attempts no tumour antigens have yet been identified. Only in one case an idiotypic derived peptide has been found to trigger CTL of the respective patient. The identification of natural antigens requires the cultivation of large amounts of tumour cells in vitro, which has been possible in two exceptional cases. The identification of synthetic epitopes for tumour specific CTL with random peptide libraries can overcome this limitation and is a powerful tool for application in the development of immune therapies for a wide range of patients.

Methods: The critical aminoacids for the construction of epitopes for the CTCL specific CTL, clone My-La CTL, were determined with synthetic peptide libraries in positional scanning OX8 format in a standard 3H-chromium release assay. 16 different peptides could be synthesised from the combinatoric of these aminoacids with the canonical anchor aminoacids for MHC binding. These peptides were tested for their capacity to stimulate My-La CTL and PBMC of an HLA-matched CTCL patient.

Results: A synthetic epitope could be identified for My-La CTL which is recognised in a HLA-restricted manner. The response towards this epitope is comparable to the response towards their natural target My-La. Taking these synthetic epitopes, T cells of a HLA-matched patient could be induced in vitro and led to the establishment of different cell lines and clones. Some of these lines recognise the peptides as well as the allogeneic but HLA-matched tumour cell line My-La, indicating that they are specific for a naturally expressed tumour antigen.

Conclusions: The identification of synthetic epitopes for tumour specific CTL clones can be used for the development of vaccines for immune therapies.

4. Biology II
5. Biology of Germinal Centers

GERMINAL CENTRE PERSISTENCE REQUIRES T CELL-DRIVEN DIFFERENTIATION OF CENTROCYTES TO CENTROBLASTS
Ian C. M. MacLennan, Carola García de Vinuesa and Montserrat Casasnovas-Palleja
University of Birmingham, Medical Research Council Centre for Immune Regulation Birmingham B15 2TT UK

A small proportion of the B cells blast recruited into T-dependent antibody responses colonize follicles where they grow exponentially to form germinal centers. When the B blasts fill the follicle centre they migrate to the pole of the follicular dendritic cell network nearest the T zone and differentiate into centroblasts. Hanna's 3H-thymidine pulse chase experiments show that centrocytes of the light zone of germinal centres arise through differentiation from centroblasts. Evidence will be presented that indicates the exponential growth and differentiation to centroblasts does not require the presence of T cells. It is now clear that an Ig V-region-directed hypermutation mechanism is active in centroblasts and centrocytes appear to be selected first on their ability to bind antigen — normally held on follicular dendritic cells — and secondly to present processed antigen to T cells in germinal centres. Kepler, and Perelson argued on mathematical grounds that the rate of acquisition of somatic mutations in the memory pool could only be achieved by the cyclic re-entry of B cells into germinal centres. Data will be presented which show that human tonsillar centroblasts can be induced to differentiate into centroblasts by cognate interaction with autologous germinal centre T cells. Further evidence will be presented and cited that indicates that this differentiation is essential for maintenance of germinal centres. These data indicate that centroblasts do not behave like the proliferating cells in epithelia, which are self-renewing. Rather the progeny of centroblasts are only both that the centroblasts of the dark zone are lost through this differentiation process in about a day. This implies that centroblast go through fewer than 4 cell divisions before becoming centrocytes. It will be important to determine if this physiological cycle of centroblasts to centrocytes followed by T-cell-induced reversion to centroblasts is a characteristic of mixed centroblastic/centrocytic lymphomas. The analysis by Thorborne's group of supernatant nylon adherent follicular lymphomas in SJL mice suggests that this might be the case.

ROLE OF BCL-6 IN GERMINAL CENTER DEVELOPMENT
Riccardo Dalla-Favera
Department of Pathology, Columbia University, New York, NY 10032

The BCL-6 gene encodes a transcriptional repressor characterized by six Kruppel-type zinc-finger domains involved in specific DNA-binding and a POZ domain involved in transcriptional repression (Ye et al., 1995; Chang et al., 1996). The BCL-6 protein is expressed at high levels in both B cells and CD4 T cells within germinal centres (GC) while it is not expressed in pro-GC B cells and in plasmacells (Cattoretti et al., 1995). BCL-6 is specifically required for GC formation since mice deficient in BCL-6 (BCL-6/-) display normal B cell, T cell and lymphoid organ development, but fail to form GC; as a consequence, BCL-6/- mice have a selective defect in T cell-dependent antibody responses including a complete lack of affinity maturation (Ye et al., 1997). Recent evidence suggest that BCL-6 is involved in three alternating pathways that regulate GC development: (i) IL-4. BCL-6 can bind a subset of STAT-6 DNA binding sites and modulate transcription activated by STAT-6, the main nuclear effector of IL-4 signaling. This function involves the modulation of Igx switching via modulation of STAT-6 dependent or steric transcript expression, as demonstrated by the fact that BCL-6 mice develop an hyper Igx response that is completely reverted in BCL-6/-/STAT-6/- mice; (ii) CD40. Signaling by CD40 and by EBV LMP-1, a CD40 functional analog, downregulates BCL-6 expression, an event that is required for CD40 induced upregulation of CD23; (iii) B cell receptor (BCR). Activation of BCR signaling via anti-μ treatment of B cell lymphomas leads to downregulation of BCL-6 expression via its MAPK-mediated phosphorylation followed by degradation by the ubiquitin-proteasome pathway (Niu et al., 1998). The mechanism by which BCL-6 regulates GC development remains unknown, although initial evidence indicates that it can prevent apoptosis. In normal GC B cells, the BCL-6-5' non-coding region is targeted by the IgV hypermutation mechanism, although a functional role for BCL-6 mutation has not been elucidated (Migliazza et al., 1995; Pasqualeci et al., 1998). The significance of these findings for normal GC development and lymphomagenesis will be discussed.

AN IN VITRO MODEL FOR CHROMOSOMAL TRANSLocations DRIVEN BY THE VDJ RECOMBINE
Kaiyong Yang *, Marco Davila*, and Gerrett Kelsoe
Department of Immunology, Duke University Medical Center, Durham, North Carolina 27710 USA

A substantial fraction of B cell leukemias and lymphomas appear to arise by chromosomal translocations. Such translocations generate oncogenic phenotypes by a variety of mechanisms including the unregulated activation of cellular protooncogenes, creation of fusion proteins with oncogenic potential, or the impairment of cell-cycle control. In B lymphomas and leukemias, many of these chromosomal translocations appear to arise by the illegitimate activity of the RAG1/RAG2 recombinase. Furthermore, the cellular phenotype and somatic genotype of such lymphomas and leukemias suggest that these illegitimate rearrangements occurred in the germinal center phase of B cell development rather than during primary lymphogenesis. VDJ recombination in germinal center B-lymphocytes appears distinctive in that the normal rules of allelic exclusion are ignored. It is not known if this represents altered control of recombinase activity or the consequence of the prolonged exposure of centrocytes to RAG1 and RAG2 proteins. In an attempt to study extended recombination activity during growth arrest, we have employed the pre-B cell line, 103/hcl-2. This line has been transformed by a temperature-sensitive (ts) Abelson murine leukemia virus, and at 350 C, 103/hcl-2 cells proliferate in vitro and express low levels of RAG1 and RAG2 mRNA and undetectable levels of RAG proteins. However, when cultures are shifted to 39° C, the ts-v viral protein is denatured, cells exit cycle and upregulate RAG1 and RAG2 and VDJ recombinase. Our analysis by chromosomal painting and LM-PCR indicate that in addition to v-EBV rearrangements at the immunoglobulin k and l loci, 103/hcl-2 cells in growth arrest acquire a surprisingly high frequency of chromosomal translocations and active VDJ recombination at the TCR loci. These preliminary observations indicate that prolonged exposure to RAG1 and RAG2 can result in double-stranded DNA breaks at many sites within the genome. Thus one of the consequences of extended cell cycle arrest in germinal center centrocytes may be the creation of recombinogenic DNA fragments and the resulting translocation of chromosomes.

*Contributed equally to this work.

BCR-INDUCED DEATH OF BL60 CELLS: A MODEL OF DEATH OF GERMINAL CENTER B CELLS
Peter H. Krammer, Axel Bouchon*, Henning Walczak
German Cancer Research Center, Heidelberg, *Basel Institute for Immunology

Antigen-induced croslinking of immunoglobulin on the surface of B cells can lead to apoptosis. This process serves to deplete the immune repertoire of anti-self specificities leading to central and peripheral tolerance of B cells. However, the mechanism of B cell receptor (BCR)-mediated apoptosis is widely unknown. By using the human Burkitt lymphoma cell line BL60 as a model system for germinal center B cells we show here that BCR-mediated apoptosis requires transcriptional activity but is neither mediated via known death-receptor systems (CD95, TNF-R1/2, TRAIL-R1/2, TRAMP) nor does it involve initial activation of caspase-8. Moreover, during BCR-induced apoptosis cytochrome c release and mitochondrial Permeability Transition (PT) precede activation of caspase-3 and -8. Although caspase inhibition after BCR-stimulation blocks cleavage of the caspase-3 substrate poly(ADP-ribose) polymerase (PARP) and DNA fragmentation it does not prevent dissipation of the mitochondrial transmembrane potential (DYN), cytochrome c release and cell death. Thus, BCR-mediated apoptosis is initiated by the caspase-independent induction of mitochondrial PT resulting in release of cytochrome c and subsequent activation of caspase-9, downstream caspases and apoptosis.