bjh review

B cells behaving badly

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Summary

The pathogenesis of B-cell lymphoproliferative disorders in general and B-cell chronic lymphocytic leukaemia in particular appears to involve dysfunctional regulation of humoral and cellular immunity with the subsequent development of genetic aberrations in B cells. In theory, either component may arise *de novo* or may be influenced by environmental exposures including infectious agents, antigens, genotoxic chemicals, or radiation. As an intermediary within the exposure-disease continuum, monoclonal B-cell lymphocytosis may be a helpful biomarker for teasing out these various contributions to risk. This article introduces a series of papers that resulted from an International Workshop held in May 2007 entitled '*Monoclonal B-cell Lymphocytosis and Chronic Lymphocytic Leukemia: Environmental and Genetic Risk Factors*'. Research efforts, such as those described in this issue, should lead to improved interventions, more predictive biomarkers, more effective treatments, and a greater appreciation of how the immune system functions over the entire human lifespan.

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The B-cell lymphoproliferative disorders (BLPD) are enigmatic disorders that have long attracted the attention of haematologists, immunologists and epidemiologists. The clinical spectrum of BLPD ranges from asymptomatic expansion of a B-cell clonal population to frank life-threatening B-cell malignancies that include non-Hodgkin lymphoma, multiple myeloma (MM), and B-cell chronic lymphocytic leukaemia (CLL). The early stages of BLPD may be detectable in peripheral blood through the appearance of monoclonal B-cell lymphocytosis (MBL) or the monoclonal antibody they sometimes secrete, as in monoclonal gammopathies of uncertain significance (MGUS). On the other hand, occult B-cell monoclonal expansions may remain hidden in the lymphatic tissues until clinical symptoms bring them to attention.

Despite the large array of genetic and phenotypic biomarkers derived from our growing understanding of B-cell development, debate continues over the origin, classification, aetiology, natural history and prognosis of BLDP. Although the lifetime risk of dying from a malignant BLDP approaches 1·5% (Ries *et al*, 2007), public health professionals can offer no advice on how to prevent them. Despite new treatments aimed at specific molecular targets for the different BLPD malignancies, cure remains elusive. Advances in preventive and therapeutic modalities will depend on a better understanding of the pathobiology of these disorders and the biomarkers that define them.

This special issue of the British Journal of Hematology is centred on CLL, a common but poorly understood and generally incurable form of BLDP. The articles reflect the proceedings of an international workshop held in May 2007 (Silver Spring, MD, USA), where physicians, laboratory scientists and epidemiologists gathered to revisit CLL in light of the most recent discoveries about its pathogenesis. Two major considerations prompted the workshop: renewed questions about environmental risk factors (Rothman *et al*, 1997;

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Richardson *et al*, 2005; Shim *et al*, 2007; Silver *et al*, 2007), and increased attention to MBL as a precedent condition (Rawstron *et al*, 2002, 2004; Marti *et al*, 2005; Shim *et al*, 2007). The workshop, which reprized a gathering held 12 years earlier, was convened to reassess laboratory and epidemiologic strategies for determining the aetiologies of BLPD. Although the focus was on methods and findings, these discussions necessarily raised related issues concerning costs and the ethical treatment of study participants. The workshop attendees also considered how to advance our understanding of environmental risk factors and pathogenesis from murine models of BLPD.

The articles that follow are grouped into four broad categories: aetiology, genetics, MBL and MGUS. Each category is introduced by an article that provides an overall perspective, followed by original reports and reviews. While the workshop topics did not include the clinical managements of CLL patients, some discussions on treatment advances have been included.

Aetiology session

The aetiology session first addressed the pathology and descriptive epidemiology of CLL. Potential aetiologic factors were critically evaluated, including ionizing radiation, non-ionizing radiation and chemicals. Methodological and other issues affecting our understanding of aetiology were also considered (Linet *et al*, 2007).

1 Classification: Classification issues are difficult to resolve in the absence of a comprehensive understanding of pathogenesis. The most recent World Health Organization (WHO) haematopoietic disorder classification combines CLL and small lymphocytic lymphoma (SLL); however, our growing understanding of pathogenesis, molecular and genetic features and epidemiological characteristics is likely to lead to further modifications in the classification of CLL and SLL.
2 Reporting: Implementation of the WHO classification scheme makes it feasible to compare incidence rates for CLL, SLL and CLL/SLL across population-based cancer registries. The notable delay in reporting of CLL, as documented for the first time in a population-based cancer registry, makes it difficult to interpret CLL incidence trend patterns for a given registry, and may also hinder attempts to compare incidence patterns across registries. Case ascertainment from death certificates is also hampered by reporting problems including inadequate or incomplete identification of cases or misclassification of CLL diagnoses. It is important to quantify reporting problems and implement new strategies to improve the ascertainment of CLL cases from all potential sources.
3 Radiation: Early important radiation epidemiological studies reported increases of the acute leukaemias and chronic myeloid leukaemia, but not CLL; there have been virtually no studies of SLL separate from all types of non-Hodgkin lymphoma combined. The role of ionizing radiation is difficult to evaluate from the existing literature. The absence of demonstrable association between radiation and CLL in the Japanese atomic bomb survivors (due in part to the rarity of CLL in Asian population) and in early studies of patients treated with radiation have led to failure to examine this outcome in a number of subsequent epidemiological studies. Methodological and biological difficulties continue to impede recent efforts to examine the potential aetiological role of ionizing radiation. Studies of non-ionizing radiation and CLL have been even more limited. To evaluate the possible role of ionizing and non-ionizing radiation exposures associated with risk of CLL, large populations and pooling of data across populations will be needed. Such epidemiological studies will also need to incorporate lifetime follow-up and detailed consideration of potential confounders and effect modifiers.
4 Chemicals: An excess risk of CLL has been identified in some studies of farm workers, with some investigations suggesting associations with particular agricultural chemicals. A few studies have reported elevated risks of CLL in the rubber and petrochemical industries. Most occupational studies have not identified specific chemicals that are linked with CLL, although limited evidence suggests an association with solvent exposures (e.g. butadiene and possibly benzene). These findings require further investigation in large populations and pooling of data across populations with well-defined exposures; ideally, lifetime follow-up should be pursued.
5 Autoimmunity and infections: The few early studies that focused on the possible relationship between autoimmune disorders and CLL reported inconsistent findings; larger investigations have found no consistent association between CLL and prior personal or family history of autoimmune diseases. Intriguingly, recent reports link infections with encapsulated bacteria to an increased risk for CLL, while chronic non-rheumatic valvular heart disease (which may involve long-term antibiotic treatment) appears to reduce CLL risks. The findings on infectious diseases and probable antibiotic use require replication and additional evaluation to determine whether the associations are truly causal.
6 Conclusions: The summaries and recommendations above point to the need for large-scale, focused epidemiological studies with careful exposure assessment and long-term follow-up. Future studies should validate CLL and SLL outcomes and facilitate data pooling across studies to obtain more precise estimates. Parallel investigations of postulated leukemogenic agents in appropriate animal models may provide valuable aetiologic insights. As MBL or other potential precursor disorders are identified and further characterized, it may be useful to further evaluate such markers in high risk population subgroups.

Genetics session

The genetics session considered the impact of heritable changes from the perspective of B-cell development, murine models, regulatory mechanisms, susceptibility genes and family studies. There was a major emphasis on the confluence of new genetic technologies and consortia approaches to advancing our understanding. Speakers emphasized that, despite the well-documented role of familiality in both CLL and MBL, the gene(s) accounting for the familial tendency remain unknown (Caporaso *et al*, 2007).

1 Somatic hypermutation of immunoglobulin V-genes is the single most predictive marker in distinguishing progressive CLL from the more quiescent forms, and germline forms of CLL are clearly associated with progressive disease. The association of germline CLL with phenotypic markers such as cytoplasmic ZAP-70 and CD38 are promising, even though there is an approximately 25% discordance with immunoglobulin V-gene (*IGHV*) mutational status, and technical difficulties in laboratory standardization remain.
2 Recent studies suggest that microRNAs (miRNAs), such as miRNA-16, and epigenomic influences, such as methylation and histone tailing, are as important to CLL leukemogenesis as are mutations and polymorphisms in structural genes. The processes involved include failure of mutated miRNA to regulate *BCL2*, epigenetic silencing of *DAPK1*, and epimutations because of DNA methylation. These remarkable findings provide new biomarkers for uncovering pathogenic pathways and identifying therapeutic targets. The ready availability of malignant B cells from peripheral blood provides a unique opportunity for further investigating these promising biomarkers.
3 The NZB mouse model for CLL underscores the importance of the miRNA-16 first observed in human CLL. Linkage of the *Mirn15a*/*Mirn16-1* complex and the development of BLPD in this spontaneous mouse model suggest that the altered expression of the *Mirn15a*/*Mirn16-1* is an important molecular lesion in CLL.
4 The chromosomal aberrations traditionally associated with CLL (deletions at 13q14, 11q23, 17p13, 6q and trisomy 12) remain important markers for prognosis and may help to understand the primary and secondary lesions that lead to the disease.
5 The degree of genetic diversity within the malignant B-cell clone becomes more apparent as it is explored more thoroughly. Such diversity may also be observed through variation in phenotypic markers.
6 Large scale single nucleotide polymorphism (SNP) studies have revealed several loci with statistical significance and suggestive pathways, but lack of confirmatory studies and adequately investigated pathways, as well as some methodological issues (including multiple testing), preclude a clear interpretation of the results at this time. A whole genome study of CLL has yet to be conducted and is a future priority.
7 Family studies show a strong genetic component for CLL and related BLPD. However, no germ line gene mutation accounting for a substantial proportion of familial CLL has yet been identified. Interesting candidates, such as *DAPK1*, are strongly implicated in some forms of CLL. Multiple genes are likely to be involved.
8 Stromal cells play a vital role in normal B-cell development. Further investigation into their function, including the use of animal models, will be required to determine their contribution to neoplastic transformation.
9 Linkage studies conducted to date have not provided strong evidence for loci harbouring susceptibility genes.
10 The meeting strongly endorsed consortia approaches to enhancing the size of both family and population studies. A linkage study involving over 200 families is in progress. Enrollment of families in large groups that will provide maximum scientific benefit should be strongly encouraged.

MBL session

The MBL session addressed issues of intraclonal heterogeneity and the difficulty in predicting when subjects with MBL will progress to clinically recognized disease. New insight into the role of T cells continue to emerge both in terms of increased levels of CD4–CD8 double positive T cells and a restricted T cell repertoire in MBL (Marti *et al*, 2007).

1 MBL is detectable in up to 5% of adult populations depending on their age distribution and the laboratory methods used to detect it. In the majority of MBL cases, the abnormal cells have the phenotype and chromosomal abnormalities associated with indolent CLL.
2 Multicolor flow cytometry is still the most important tool for detecting and characterizing MBL. Instruments that allow at least three-colour determinations are required for detection, but four (or more) colour analysis is optimal. It will be necessary to combine flow cytometric sorting with *IGHV* mutational analysis when multiple clones are present.
3 MBL is essential in the pathway from B-cell normality to indolent, *IGHV*-mutated CLL, but the exact relationship between MBL and CLL remains to be fully delineated. Current evidence suggests that MBL can persist indefinitely, or progress to CLL and other B-cell malignancies. Progression to CLL requiring treatment appears to occur at a rate of approximately 1% per year. MGUS provides a model for determining the natural history of MBL and demonstrates that its ultimate impact on health and longevity can only be ascertained through long-term longitudinal studies.
4 The striking relationship between age and MBL (as well as CLL) suggests that immune senescence may be a contributor to emergence of monoclonal B-cell populations. A similar age-related increase in MBL occurs in mice, even in strains that are not prone to spontaneous BLPD. Immune senescence may be related to the acquisition of certain persistent infections, such as Hepatitis C, or to increased

reactivity to autoantigens, such as rheumatoid factor. T-cell clonotypic expansions, particularly those in which peripheral blood T cells express both CD4 and CD8, may contribute to immune senescence and may occur with or without the appearance of MBL.

5 Intraclonal heterogeneity is a frequent occurrence in both MBL and *IGHV*-mutated CLL. How this predicts progression of MBL to CLL is not clear at this time. Although it is frequent, intraclonal variation is generally restricted to only 1- or 2-point mutations in each sequence and requires extensive laboratory effort to uncover. However, subcloning of the conventional polymerase chain reaction product and or flow cytometric sorting and molecular analysis will be required in some cases.

6 MBL maybe a useful biomarker in looking for early adverse effects in environmentally exposed populations. Standardization of the laboratory methods used to detect it will be required for comparing data, examining trends, evaluating effects of covariates, understanding rates of progression and establishing prognostic markers. The costs and ethical issues surrounding such studies are impediments that must be overcome to make them feasible.

Monoclonal gammopathies of uncertain significance

This session broadened the focus to consider MGUS, the humoral counterpart to MBL. Features of these biomarkers and their respective disease endpoints (MM and CLL) were compared and contrasted, including racial disparity patterns, familial aggregation and the relation to infectious disease and autoimmunity.

The final speaker was Dr Robert A. Kyle, who presented results from his extensive longitudinal studies of MGUS and smoldering MM. These investigations have added to our understanding of the natural history of this condition and clarified the predictive biomarkers for progression to malignancy (Vogt & Marti, 2007).

Overall recommendations

1 Future studies of environmental exposures and CLL should take into account the fact that CLL is a BLPD and shares a common cellular origin with MBL, Waldenstrom macroglobulinaemia, non-Hodgkin lymphoma and MM. Molecular and cellular biomarkers may refine traditional disease classifications and provide new insights into molecular origins of the various BLPD. Controversy still persists about whether CLL and SLL should be considered as one disorder.

2 The laboratory methods used to characterize BLPD should be standardized through international, multi-centre collaborations that include shared samples and protocols. To that end, existing electronic data sets could be compiled from previously defined cases of MBL and used as a starting point for such standardizations.

3 A multidisciplinary approach, including clinicians, geneticists, epidemiologists, haematopathologists and statisticians, should be used to design, analyse and interpret data from future studies.

4 Animal models of BLPD should be combined with the exposure models used in risk assessments, such as those in the US National Toxicology Programme. In particular, the various mouse models of CLL, starting with NZB, should be used to identify environmental exposures that increase risk of disease in susceptible strains.

5 An international consortium that can pool MBL cases from existing and ongoing studies should be strongly encouraged. This group would also undertake standardization of diagnostic and research methods to be used in MBL research.

6 Because the definitions of CLL and MBL are likely to be refined as our understanding evolves, studies looking at risk factors, incidence and prevalence, natural history, and therapeutic interventions should be interpreted in light of the definitions used for the particular study and taken into account in cross-study interpretations.

Dedication

The workshop organizers dedicate this special issue to Dr Robert A. Kyle at the Mayo Clinic College of Medicine. Dr Kyle was kind enough to share the insightful perspectives gleaned from the pioneering work he has conducted throughout his remarkable career in medicine and biomedical science. His exemplary presentation and his comments during discussions focused the entire workshop on the need for diligent follow-up to unravel the natural history of MBL. The graphs he used to illustrate MGUS and smoldering MM provided a clear roadmap as where we have to go with MBL. And along with the scientific rigor he brought to the workshop, he addressed the ethical treatment of study participants and patients in a way that clearly reflected his compassion and dedication as a physician.

Disclaimers

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centres for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. This work does not represent the official position of the Food and Drug Administration.

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