Murine models for chronic lymphocytic leukaemia

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Abstract
CLL (chronic lymphocytic leukaemia) is characterized by the clonal outgrowth of B-lymphocytes with the distinctive phenotype: CD19⁺CD5⁺CD23⁻IgM⁺. These malignant B-cells accumulate in the PB (peripheral blood) and lymphoid organs, and are generally arrested at the G₀/G₁-phase of cell cycle and display a resistance to apoptosis. To date, most of the CLL research has been carried out using PB samples obtained from patients with established CLL, which have proved instrumental in characterizing the disease. However, while CLL cells appear to have a defect in apoptosis in vivo, they rapidly undergo apoptosis ex vivo, suggesting that CLL cells are dependent on microenvironmental signals to enhance cell survival. One approach used to define the cellular and molecular events that govern CLL has been the development of murine models that replicate the human disease. As well as providing a deeper understanding of the potential triggers for CLL, these models provide preclinical in vivo systems to test novel therapies. The focus of the present review will be to highlight the recent advances in the development of mouse models for CLL.

Introduction
CLL (chronic lymphocytic leukaemia) is the most common leukaemia in the Western world for which there is currently no cure. CLL is characterized by the accumulation of long-lived B-cells that display a resistance to apoptosis and have undergone cell cycle arrest in the G₀/G₁-phase [1]. The clinical course of CLL varies significantly, as some patients survive over a decade with stable disease requiring little or no treatment, while in others the leukaemia behaves aggressively, with survival measured in months despite treatment [1,2]. Intensive studies have established a variety of mechanisms potentially responsible for the development of CLL, including a number of distinct chromosomal abnormalities and intrinsic defects in their apoptosis machinery due to higher levels of the anti-apoptotic protein Bcl-2 family member proteins Bcl-2 and Mcl-1 [2], thus making this disease extremely heterogeneous. Over the past decade, CLL has been broadly subdivided into two prognostic subsets, by virtue of the mutational status of the Ig (immunoglobulin) VH (variable heavy chain) genes. CLL cells carrying <2% divergence from germline VH sequences [U-CLL (unmutated CLL)] are generally associated with poor prognosis compared with cells carrying mutated VH genes [M-CLL (mutated CLL)] [3,4]. Surrogate markers that tend to correlate with the U-CLL subset include ZAP-70 (ζ-chain [TCR (T-cell receptor)]-associated protein kinase of 70 kDa) and CD38 expression, and certain cytogenetic abnormalities such as deletions in chromosome 17 leading to aberrations of the p53 tumour suppressor gene [2]. Despite this progress, the cause of CLL remains elusive and no single genetic event has been identified. Therefore a deeper understanding of the biology of CLL is required to identify novel treatment strategies.

Murine models of CLL
It has become clear in recent years that CLL is a more dynamic disease than previously considered. Therefore rather than malignant B-cell clones accumulating over time, it is now understood that there is a turnover of CLL cells and an elevation in proliferation cycles can be related to disease progression [5]. Thus, in addition to the in vitro cultures that are used to study CLL, mouse models that develop leukaemias resembling human CLL will provide a powerful systems approach to investigate the dynamic nature of this disease.

TCL-1 (T-cell leukaemia/lymphoma-1)
TCL-1 is a proto-oncogene that has been functionally linked to the enhancement of Akt-mediated signalling pathways [6]. Tcl-1 expression pattern in normal B- and T-lineage cells is similar in human and mouse. While Tcl-1 is only expressed at the early stages of T-cell development, Tcl-1 expression is initiated at the pro-B-cell stage and can be detected in MZ ( marginal zone) (B220⁺CD21⁽b⁾CD23⁻) and follicular (B220⁺CD21⁽b⁾CD23⁽b⁾) B-cells, before being extinguished in post-GC (germinal centre) memory B-cells and plasma cells [7–9]. Tcl-1 was initially implicated in T-cell leukaemias as T-cell prolymphocytic leukaemias were found to have a chromosome translocation bringing the TCRα/8 or β locus into close proximity to TCL-1, resulting in an up-regulation of Tcl-1 [9]. More recently, overexpression of Tcl-1 has been...
noted in a number of B-cell leukaemias and lymphomas in the absence of chromosome translocations [8] prompting the development of mouse models that overexpress hTCL-1 (human TCL-1) in B-lineage cells. The introduction of a transgene encoding hTCL-1 under the control of the IgH (Ig heavy-chain gene locus) Eμ enhancer resulted in the overexpression of Tcl-1 at the immature and mature stages of B-cell development. Analysis of the mice revealed an expansion of B220+IgM+CD5+CD11b+ cells, initially in the peritoneal cavity, then later in the spleen, bone marrow and PB (peripheral blood), which in older mice (13–18 months) possessed a number of features similar to human CLL [10]. In keeping with this finding, Tcl-1 expression is detectable in most of the CLL patient samples, although a subset of cases (∼10%) is negative or only weakly positive for Tcl-1. Higher expression levels of Tcl-1 are associated with the poor prognostic U-CLL and ZAP-70+ subsets, as well as samples that have an 11q22 deletion, which is associated with the loss of ATM (ataxia telangiectasia mutated) [11]. Recently, it was determined that the miRNAs (microRNAs), miR-29 and miR-181 are generally inversely correlated with Tcl-1 expression in the CLL samples, suggesting that Tcl-1 expression in CLL is, at least in part, regulated by miR-29 and miR-181 [12].

In normal human and mouse B-lymphocytes, the B-cell repertoire is made up of B-cells expressing random VH genes. However, in both U-CLL and M-CLL cases, preferential usage of specific VH genes has been noted [4,13,14]. Analysis of the IgH gene locus in Eμ-TCL-1 mouse-derived B-cells indicated minimal levels of somatic mutation, suggesting that this model may represent the more aggressive subset of human CLL, U-CLL [15]. Interestingly, a bias towards the use of VH11, VH12 and VH4 gene segments was noted, suggesting that the expanded CD5+ population present in Eμ-TCL-1 mice was derived from B-1a lineage cells [10,15]. It has been hypothesized that CLL may be derived from MZ B-cell populations in humans [16], which may bring into question the relevance of mouse models that display a B1a-derived CLL-like disease. However, studies suggest that MZ B-cells in humans are similar to both B1- and MZ B-cells derived from mice, suggesting that in mice these lineages converge [17].

In contrast with the Eμ-TCL-1 transgenic line described above, an additional line of Tcl-1 transgenic mice has been described in which hTCL-1, cloned from an AIDS-related B-cell lymphoma, was placed under the control of the Eμ-T29 promoter, thus allowing overexpression of TCL-1 in both B- and T-lineage cells. While lymphocyte populations appeared normal in young TCL-1-Tg mice (3 months), most mice between 7 and 13 months had elevated lymphocyte counts and spleen weights. While most of the Eμ-B29-hTCL-1 mice had polyclonal LPDs (lymphoproliferative diseases) in both B- and T-lineages, some had an expansion of monotypic B-cells that had an activated IgM+ B220+CD5+ mature B-cell phenotype differing from the B-cell expansion that developed in Eμ-TCL-1 mice. Indeed, the mice developed different types of lymphomas including diffuse large B-cell lymphoma, Burkitt-like lymphoma and follicular lymphoma. Molecular analyses of the IgH gene locus revealed that these B-lineage cells had undergone somatic hypermutation and strongly expressed Bcl-6, suggesting that Tcl-1 overexpression in these Eμ-B29-TCL-1 mice gives rise to post-GC-derived B-cell lymphomas [18].

There are clear differences in the diseases that arise in the two hTCL-1 transgenic models, resulting in the transformation of either B-1 (Eμ-TCL-1) or post-GC B2- (Eμ-B29-TCL-1) cells. This may be due to the different promoters that were used in generating these mice, the former CLL model overexpressing Tcl-1 only in B-lineage cells, while the latter resulted in the expression of Tcl-1 in both B- and T-cells. However, both models display a long latency prior to B-cell transformation, suggesting that additional mechanisms are required for leukaemogenesis.

**New Zealand mouse strains**

NZB (New Zealand Black) and NZW (New Zealand White) mouse strains present a classical model for autoimmune disease resembling SLE (systemic lupus erythematosus); however, due to their expansion of CD5+ B1-cells, these mouse strains also provide a model of B-cell malignancies [19,20]. The LPD that develops in the NZB and NZW mice has been reported to resemble human CLL, as it is characterized by a late onset outgrowth of clonal IgM+ B220+CD5+ B-cells [19,20]. It has been determined that related MHC haplotypes predispose NZW mice to develop either SLE or CLL, with the H-2d homozygous mice acting as an element to promote the development of CLL [20,21]. NZB mice, while carrying the H-2d haplotype, do present a clonal expansion of hyperdiploid B-1 cells although at lower frequency than that noted in NZW mice, suggesting that multiple genetic elements predispose the New Zealand mouse strains to develop CLL-like disease [19,22]. In addition, NZB mice have raised levels of serum IgM and produce autoantibodies against red blood cells, which lead to the development of autoimmune haemolytic anaemia, a common autoimmune complication noted in CLL patients [22,23].

A recent finding indicates a similarity between the NZB CLL-like disease and human CLL at the molecular level. Lesions for hyperdiploid LPD in NZB mice were localized to chromosomes 14, 18 and 19 [24]. Mouse chromosome 14 is syntenic with the human region 13q14, which is either hemizygous or homozygously lost in approx. 50% of CLL cases [25,26]. Interestingly, miR-15 and miR-16 miRNAs, which target Bcl-2, are located in this region and are found to be deleted or down-regulated in CLL [26–28]. Closer analysis of chromosome 14 in NZB mice identified a point mutation, which resulted in a decrease in miR-16. When miR-16 was introduced back into the CD5+ CLL-like cells, elevated levels of apoptosis were noted [24]. This finding not only identifies a potential lesion for the development of CLL, but also highlights a novel avenue for therapy [24,27]. Also the NZB mouse provides a model for studying a CLL-like disease in an environment of autoimmunity, which is an important aspect of the human disease, particularly in light of the emerging model, which considers that the cell of origin
may be an antigen-experienced mature B-cell stimulated by autoantibodies [16,29].

**Bcl-2 x TRAF2DN mouse**

Bcl-2 is overexpressed in most of the CLL cases and increases in Bcl-2 expression have been related to increasing chemoresistance in CLL cells [30]. However, overexpression of Bcl-2 alone in mouse B-cells is not an initiation factor for a CLL as it generated a polyclonal expansion of B-lineage cells in the absence of leukaemogenesis [31]. Similarly, mice expressing a transgene encoding a dominant-negative TNF receptor (TNF (tumour necrosis factor) receptor), TRAF2 (TRAF-2-associated factor 2) (TRAF2DN), resulted in a polyclonal expansion of B-cells, suggesting that inhibition of TRAF2 confers apoptosis resistance on B-cells [32]. However, when double transgenic mice were generated overexpressing Bcl-2 and TRAF2DN, most of the older mice (6–14 months) developed a clonal CD5+ B-cell leukaemia (B220+IgMhiIgDloCD21hiCD23−CD11b−) that resembled human CLL [33]. These mice displayed a reduced life span compared with the single transgenic mouse lines. Notably TRAFs, and in particular TRAF1 (a natural inhibitor of TRAF2), are elevated in CLL cells [34]. Interestingly, B-cells derived from the Bcl-2 x TRAF2DN mice failed to apoptose in response to dexamethosone- and fludarabine-treatment, indicating that these mice may provide a model for testing novel drugs targeted towards chemoresistant CLL.

**APRIL (a proliferation-inducing ligand) mouse model**

BAFF (B-cell activating factor) from the TNF family and APRIL are members of the TNF family that are found to be elevated in the sera of CLL patients, aiding in CLL cell survival in vivo [35]. Nurse-like cells that are present in the microenvironment of the lymphoid organs as well as the CLL cells themselves act as a source of BAFF and APRIL [36,37]. In an effort to address whether APRIL was sufficient to initiate CLL, APRIL was overexpressed in T-cells, which led to the secretion of APRIL and a subsequent increase in serum levels within the transgenic mice. A mild expansion of CD5+ B1a-cells was noted over time due to an increased survival capacity of B1-cells in the presence of APRIL rather than increased proliferative potential [38], possibly via signalling through the classical NF-κB (nuclear factor κB) signalling pathway, which is activated in response to APRIL in human CLL cells [39]. However, these mice did not die prematurely, suggesting that additional components need to be deregulated to generate a CLL-like disease.

**PKCoα (protein kinase Cα)-KR mouse**

PKC is a family of serine/threonine protein kinases that have been implicated in the regulation of survival, proliferation and differentiation of CLL cells [40]. We have recently developed a novel mouse CLL model, by stably expressing a plasmid encoding dominant-negative PKCoα (PKCoα-KR) in a foetal liver-derived HSC (haemopoietic stem cell) enriched population from wild-type mice and culturing these cells either in B-cell generation systems in vitro or in vivo [41,42]. This resulted in the spontaneous generation of a population of cells that bear hallmark characteristics of human CLL cells at the level of: (i) phenotype, CD19hiCD5+CD23+IgMloCD21+CD11b−; (ii) cell cycle phase (halted in G2/M, ex vivo); and (iii) resistance to apoptosis possibly due to an elevation in Bcl-2 and Mcl-1 transcript levels [41]. CLL cells generated in vivo accumulated in the lymphoreticular system similar to that noted in CLL patients, demonstrating the applicability of this murine CLL model to the human disease. Moreover, PKCoα-KR-expressing HSCs possessed an enhanced proliferative capacity both in vivo and in vitro, possibly reflecting the dynamic cellular kinetics that exist during the progression of human CLL [5]. Studies are currently under way to further characterize this model.

**Preclinical models for drug therapies**

To test whether these mouse models could be used as controllable and reproducible preclinical models for novel treatment, Eµ-TCL-1 mice with established CLL were treated with a first-line treatment for CLL: fludarabine. A reduction in PB lymphocyte count and spleen size was observed, which resulted in an increased survival compared with untreated animals. As frequently noted in human CLL, with time there was an emergence of fludarabine-resistant cells in vivo in these treated mice, demonstrating the potential validity of using this mouse model as a preclinical system for developing novel therapeutic treatments for CLL [43].

**Concluding remarks**

As CLL is such a heterogeneous leukaemia, it is likely that each of the described models will provide important information regarding the molecular mechanisms that mediate the initiation and maintenance of CLL. Indeed, these models have already drawn parallels with human CLL by highlighting the role of miRNAs in controlling the expression of Bcl-2 [24,27], as well as illuminating critical factors in forming appropriate CLL-promoting microenvironments to enhance cell survival. Therefore each of these novel systems provides a powerful tool to investigate individual aspects of cellular and molecular mechanisms that govern the initiation and maintenance of CLL and provides a preclinical system to test novel drug therapies.

**References**
