Light chain amyloidosis results from the misfolding and aggregation of an immunoglobulin light chain usually produced by clonal plasma cells in the bone marrow. Hence, light chain amyloidosis is both a cancer and an amyloid disease and is the most rapidly fatal of the systemic amyloid diseases. Patients with light chain amyloidosis are usually treated with chemotherapy agents to eradicate the plasma cell clone. However, the toxicity of these drugs in the background of proteotoxicity caused by the process of light chain amyloid fibril formation (amyloidogenesis) often limits how much of the chemotherapeutics can be given. Several experts have hypothesized that if amyloidogenic light chain secretion and/or light chain amyloidogenesis could be blocked, the associated organ toxicity would be ameliorated, enabling more aggressive and effective chemotherapy regimens to be used.

While transgenic cell and murine models are now available for nearly every human amyloid disease, these have proven elusive for light chain amyloidosis despite significant effort on the part of several laboratories. Light chain amyloidosis mouse models have been hard to generate probably because of the severe cytotoxicity and organ toxicity associated with the process of light chain amyloidogenesis—leading to embryonic lethality. Here, Ward et al report the long-awaited first transgenic murine model of light chain amyloidosis.

The amyloidogenic light chain light chains in the 3 lines generated are comparable with nonamyloidogenic light chain light chain levels found in healthy human adults. Because the efficiency and rate of amyloid formation is dependent on the concentration of amyloidogenic light chain, the low plasma concentration of amyloidogenic light chain minimizes amyloidogenesis, which is probably why these mouse lines could be generated. It is envisioned that these mice will be very useful for evaluating proteostasis regulator candidates that selectively lower amyloidogenic light chain secretion without altering proteome secretion in general, including antibody secretion. Moreover, Ward et al showed that all 3 mouse lines produce amyloid in the lumen of the gastric glands of the stomach. The acidic environment of the stomach probably partially unfolds the destabilized light chain, which then forms a conformational intermediate that misassembles, leading to a dysplastic stomach epithelium and dilated glands filled with light chain amyloid. Approximately 20% of the transgenic mice exhibited a neurodegenerative phenotype reflected by a gait disturbance and limb clenching when the mice were picked up by the tail, and these mice demonstrated impaired inclined treadmill performance.

Ward and colleagues beautifully demonstrated that these mice could be used to assess the efficacy of anti-light chain amyloid drug candidates. Transgenic mice 3 to 6 months of age were treated with doxycycline in the drinking water. After 7 months of treatment, 23% of the mice had stomach amyloid detected by Congo red versus 69% of the untreated group. While the mechanism of doxycycline action merits further investigation, what is clear is that this murine model is useful for testing antiamyloid agents.

Like almost all “first transgenic disease models,” this is not the ultimate murine model in that amyloidogenic light chain expression is low and amyloidogenicity appears to require the acidity of the gastric gland to occur. However, this model appears to be superior to the nontransgenic mouse models. These include a model introduced by Pepys and colleagues wherein they repeatedly injected human light
chains from light chain amyloidosis patients into mice to observe amyloidogenesis of the human protein. This probably could be done in immune-compromised mice for longer-term experiments to avoid the immune system response if sufficient amounts of amyloidogenic light chains could be procured, which is probably best done by recombinant expression. Ward and coworkers also previously published a nontransgenic light chain amyloidosis model wherein plasmacytoma cells stably transfected with an amyloidogenic light chain are injected into mice, allowing short-term siRNA proof-of-principle experiments to be conducted. It seems that the next generation of mouse models would include those where the production of the amyloidogenic light chain could be turned on at relatively high levels after the mice reach adulthood—simulating what occurs in human light chain amyloidosis.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

LYMPHOID NEOPLASIA

Comment on Fazi et al, page 6618

MBL: mostly benign lymphocytes, but…

Gerald E. Marti NHLBI

In this issue of Blood, Fazi and colleagues have asked important questions and added several new observations to our understanding of monoclonal B-cell lymphocytosis (MBL) in its role as the precursor state of chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL/SLL). First, it is important to note the role of the absolute B-cell count in distinguishing low-count, population-screened MBL from clinical MBL with lymphocytosis. Low-count MBL can be designated by a clone size of 50 B cells per µL or less, while clinical MBL occurs with a B-cell lymphocytosis of 1500 cells per µL or greater. Rawstron et al have an excellent diagram showing the difference between low-count and clinical MBL (see Figure 12). The top panel in the figure here is a modification of the Rawstron diagram. Second, there are 3 common variants of MBL. They are CLL-like MBL (CD5 + CD20 dim), atypical MBL (CD5 + “bright CD20”), and CD5-negative MBL. (See Vogt et al1 and Marti et al1 for examples of these patterns; the bottom panel in the figure here is from this author’s former laboratory.) In a landmark flow cytometric analysis paper, the Primary Health Care Group of Salamanca provided data confirming that the prevalence of MBL is age dependent and 100-fold in excess of CLL. Why this large difference, and what are the molecular and biologic features that favor progression to CLL?

Briefly, this longitudinal study by Fazi et al is a re-evaluation, follow-up study of population-based, low-count MBL. The investigators had previously found 137 cases of MBL in this genetically isolated rural Italian population. Seventy-six individuals were available for re-evaluation with a median follow-up of 34 months, or almost 3 years. The majority of cases are CLL-like MBL but also include atypical and CD5-negative MBL. Ninety percent of CD5 + CLL-like MBL cases show no progression and are stable. Ten percent of cases could not be confirmed in follow-up; these were originally at the lowest threshold of detection when first identified. There was no statistically significant increase in white blood cell count, absolute lymphocyte count, or clone size from baseline. A greater number of the atypical and CD5-negative cases could not be confirmed at repeat testing. It would now seem that low-count CLL-like MBL can be referred to as persistent MBL and would require no further evaluation. Long-term studies in a blood-donor setting would allow for confirmation of this finding of persistence without progression as well as the transient nature of atypical and CD5-negative MBL.

The second major finding reported by Fazi et al concerns the cytogenetic analysis of MBL. Flow cytometric cell sorting followed by slide-based FISH data are provided on these cases of low-count MBL. They show that 50% of cases display heterozygous and homozygous 13q14 deletions. This is the first extensive report of 13q14 deletions in low-count CLL-like MBL and it is important because, as the authors point out quite convincingly, the acquisition of the CLL-associated genomic abnormalities occur very early in the natural history of MBL/CLL. It follows then that a further event is required for leukemic progression. The Ghia laboratory has already shown that in CLL-like MBL, Ig gene use is more similar to normal B cells than CLL cells. Given that in MBL, the Ig gene mutational status is predominately mutated, it would be of interest to reanalyze one more time to ascertain if the Ig gene repertoire of 13q14 del MBL is similar to that found in CLL rather than normal B cells.

The third new finding by Fazi et al shows that 80% of MBL is associated with double-positive T-cell clones (44.4% CD4highCD8low and 36.1% CD8highCD4low) using T-cell receptor Vβ8 (TRBV8; 12/27, 44.4%) and T-cell receptor Vβ8 (TRBV8; 9/27, 33.3%). These findings are new and one would only hope that this type of analysis would be extended to clinical and familial MBL. Are the T-cell clones so-called “cognate clones” associated with a given MBL clone? Does this argue the case for chronic antigen stimulation for both MBL and T-cell clones, or is it a reflection of immune-senesence?
Finally, a transgenic light chain amyloidosis mouse model

Jeffery W. Kelly