Follicular lymphoma: too many reminders for a memory B cell

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Memory B cells are a dynamic subset of the mature B cell population that in some cases can reenter germinal centers (GCs) in response to iterative infections. Such a reactivation can lead to accumulation of genetic lesions in these cells, potentially from repetitive activation of the B cell mutator enzyme AID. Normal memory B cells do not survive repeated reentries into GCs. In this issue, Sungalee et al. demonstrate that memory B cells harboring the oncogenic *BCL2:IGH* translocation, which results in constitutive BCL2 expression, survive multiple GC entries upon repetitive immunization. Through these multiple GC reentries, the hallmark *BCL2:IGH* translocation enables AID-induced hypermutation and propagates clonal evolution toward malignant follicular lymphoma.

Clonal evolution of follicular lymphomas

Follicular lymphoma (FL) is an indolent subtype of non-Hodgkin's lymphoma (1) that clonally evolves over decades (2) before presenting as overt disease. In almost all cases of FL, the t(14;18)(q32;q21) translocation, which places the oncogene *BCL2* under the control of the immunoglobulin heavy chain (*IGH*) enhancer, represents an initial genetic event (3–5). The juxtaposition of *BCL2* to the J_H cluster of the *IGH* locus confers a survival advantage on developmentally arrested germinal center (GC) B cells (6–8). Normal GC B cells do not express BCL2 and are characterized by a proapoptotic program of gene expression that includes cell surface death receptor *FAS*, tumor protein *TP53*, and BCL2-associated X (*BAX*) (9). Therefore, GC B cells are destined to die (9, 10) unless they are rescued by survival signals emanating from a B cell receptor (BCR) with high affinity to antigen. BCL2 expression is triggered in memory B cells that arise from a GC reaction and increases the half-life of these cells (11). As a consequence, transgenic mice that overexpress BCL2 in the hematopoietic compartment display a marked reduction of B cell apoptosis in GCs as compared with that seen in WT controls (12). Affinity maturation in these transgenic mice results in a memory B cell compartment with reduced stringency in the selection of high-affinity BCRs (12). These findings suggest that constitutive expression of BCL2 as the result of the t(14;18)(q32;q21) translocation fundamentally alters GC and memory B cell dynamics.

Despite constitutive BCL2 expression, cooperating genetic lesions are required for malignant transformation of B cells into full-blown FL (7, 8, 13, 14). The t(14;18)(q32;q21) translocation occurs at a low frequency in normal B cells in about 70% of healthy individuals and increases with age (15–18). Individuals in whom t(14;18)(q32;q21) translocation has occurred display developmentally arrested B cells (FL precursors) that have transitioned through the GC and have imprints of AID activity, namely somatic hypermutation (SHM) and class switch recombination (CSR) (16, 18). Full-blown FL arises from these atypical

B cells decades later (2). The long latency period observed in FL development indicates a prolonged process of clonal evolution. Such a protracted clonal evolutionary process was previously demonstrated in a subset of pre–B cell acute lymphoblastic leukemia (ALL) patients who harbor the ets variant 6 runt-related transcription factor 1 (*ETV6-RUNX1*) translocation (19–21). Although the *ETV6-RUNX1* rearrangement arises in utero (20), less than 1% of children who carry this rearrangement develop full-blown leukemia, which requires postnatal acquisition of secondary lesions in the preleukemic clone (21).

Only recently have studies begun to elucidate the mechanisms responsible for the long latency period in FL evolution. Multiple studies have shown that IgM+ memory B cells get reactivated and can reenter the GC upon antigenic recall (22–24). Importantly, GC reentry is restricted to the IgM+ memory B cell subset and does not occur in IgG+ memory B cells (22, 23). In this issue, Sungalee and colleagues reveal that the iterative GC reentry of t(14;18)(q32;q21)-carrying IgM+ memory B cells upon chronic immunization is the central driver of follicular lymphomagenesis (25).

Chronic infection drives accumulation of t(14;18) (q32;q21) B cells

Sungalee and colleagues developed murine models that recapitulate the genesis of FL by elegantly mimicking the sporadic occurrence of the t(14;18)(q32;q21) translocation in humans (25). *BCL2*tracer mice harbor an engineered human *BCL2* transgene that must undergo rearrangement to be transcribed and is highly expressed in B cells upon activation of the RAG recombinases (25). The same *BCL2* transgene could also be transduced via retrovirus into BM precursors, which could then be transplanted into irradiated mice. In humans, the t(14;18)(q32;q21) breakpoint combines features of RAG-mediated V(D)J recombination and AID-dependent CpG targeting
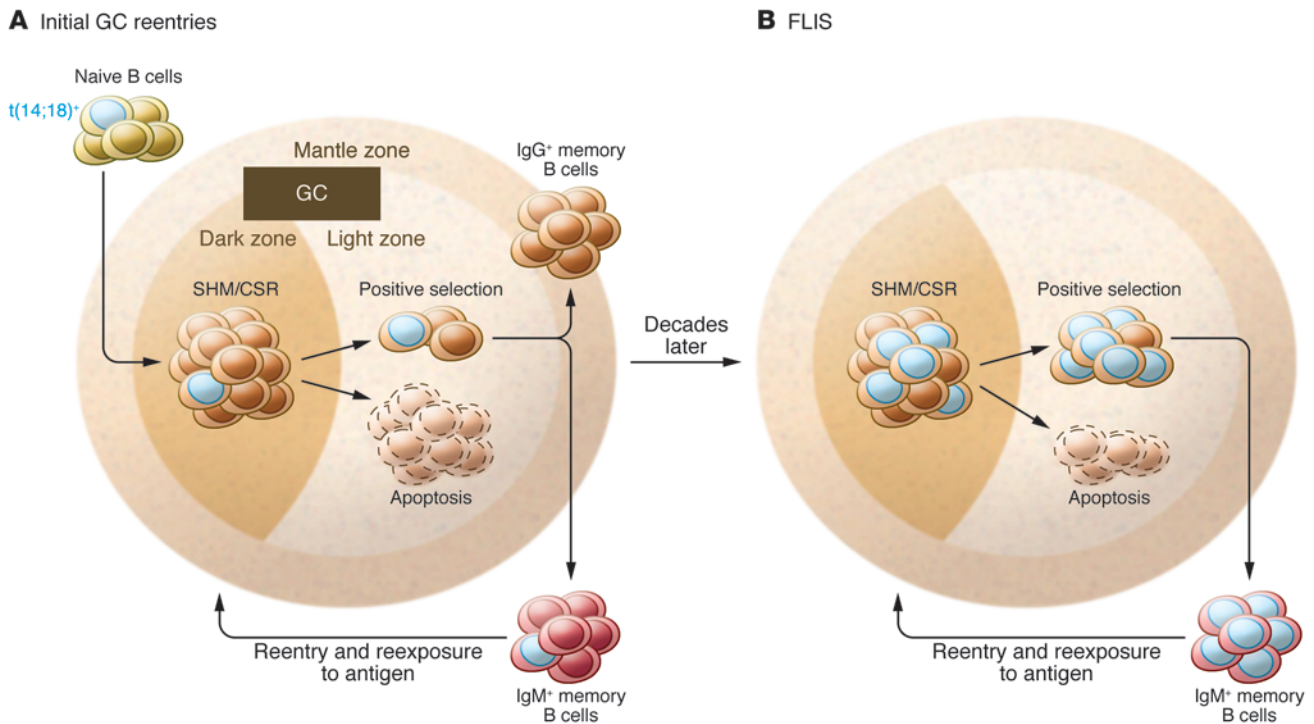
Figure 1. Memory B cell dynamics in the multistep development of follicular lymphoma. (**A**) Normal and t(14;18)[+] naive B cells both enter into GC reactions upon immunization. GC B cells undergo SHM and CSR. Owing to constitutive BCL2 expression, t(14;18)[+] cells have a survival advantage over normal GC B cells; therefore, BCL2-overexpressing t(14;18)[+] cells are positively selected into the IgM[+] memory B cell pool and are capable of GC reentry upon reexposure to antigen. With every successive cycle of GC reentry, t(14;18)[+] cells outgrow their healthy counterparts. (**B**) Decades of iterative GC reentries cause an accumulation of abnormal t(14;18)[+] cells in the GC (termed FLIS). This process produces a continuous output of t(14;18)[+] IgM[+] memory B cells, ultimately leading to overt FL.

and typically occurs in pro–/pre–B cells (26). This feature of the t(14;18)(q32;q21) translocation makes the RAG-dependent *BCL2*[tracer] model developed by Sungalee et al. physiologically relevant to human disease. Moreover, these models could be tweaked to study perturbations in the frequency of BCL2-expressing B cells in different lymphoid organs. The authors also investigated immunization-induced bidirectional transitions between GC and memory B cells in response to high expression levels of BCL2 (25).

Sungalee and colleagues determined that BCL2-expressing memory B cells are able to reenter the GC following repeated antigen challenges. As demonstrated by the *BCL2*[tracer] mouse model, a small number of initial GC transits is insufficient for the accumulation of abnormal BCL2-expressing B cells and the induction of FL (25). Therefore, the authors proposed and validated that chronic or repetitive antigen exposure is required to induce multiple iterative GC entries and genetic instability. Only BCL2-expressing memory B cells underwent repetitive GC transits by evading apoptosis (ref. 25 and Figure 1).

The authors found that these cells also failed to differentiate after GC passage and continued to express IgM on the surface, thereby continuously accumulating in the IgM[+] memory B cell pool (ref. 25 and Figure 1). The impaired differentiation, longer half-life (11), and IgM expression allowed BCL2^{hi} memory B cells to iteratively reenter the GC at multiple cycles of antigenic recall. Moreover, these features also conferred a survival advantage for these BCL2-expressing B cells compared with normal B cells in the lymphoid follicle (ref. 25 and Figure 1).

Healthy B cells were repeatedly weeded out by subsequent returns to the GC, leaving the developmentally blocked BCL2^{hi} B cells to colonize the GC and cause FL in situ (FLIS) (Figure 1). Some of these BCL2^{hi} B cells also disseminated into peripheral lymphoid tissues and were termed FL-like cells (FLLCs). Concordantly, Sungalee and colleagues observed wide dissemination of atypical t(14;18)(q32;q21)[+] B cells in lymphoid tissues from healthy individuals who had no obvious symptoms of FL (25). FLIS and FLLCs both resembled GC B cells and have a BCL2^{+}/

BCL6^{+/lo} phenotype (25). These benign precursors eventually evolve over several decades (up to 20 years) to give rise to full-blown FL (13, 27).

Repeated GC transits carry the risk of genetic instability through repetitive exposure to AID expression (28); therefore, lymphomas arising from the GC, such as FLs, display a high degree of DNA damage resulting from SHM and CSR (14, 29–31). The findings by Sungalee et al. (25) build on these observations. Using whole-exome sequencing, the authors found evidence of increased mutations, possibly resulting from AID activity, in both GCs and memory B cells overexpressing BCL2 as compared with empty vector–transduced controls. Moreover, Sungalee and colleagues interrogated their clonal evolution model in humans by comparing the intraclonal variation (ICV) in t(14;18)(q32;q21)[+] memory B cell clones with that in normal memory B cell clones. In agreement with their earlier findings, ICV was higher in the t(14;18)(q32;q21)[+] memory B cell clones with an increased rate of SHM and CSR as compared with that in normal counterparts in the same individual (25).

Thus, the t(14;18)(q32;q21) translocation supports multiple iterative reentries of IgM[+] memory B cells into the GC compartment upon chronic immunization and thereby promotes AID-induced hypermutation. Cooperation between these two processes facilitates the malignant transformation of t(14;18)(q32;q21)[+] B cells into overt FL (Figure 1).

Conclusions and future perspectives

A number of recent studies have shown that chronic and repetitive immune responses trigger reactivation and reentry of IgM[+] memory B cells into the GC (22–24). This was unexpected, because earlier studies using genetic removal of a cognate antigen demonstrated that long-lived memory B cells do not need "reminders," such as antigen persistence and reentry into GCs. Memory B cells that have evolved high-affinity antibodies are highly valuable to the adaptive immune system and therefore were thought to be exempt from GC reentry and the risk of permanent deletion from the repertoire (32). A possible explanation for this paradox lies in the coevolution of antibodies and antigens. For example, viral, parasitic, and bacterial antigens often self-mutate to evade host immune defenses (33–36). Such antigenic coevolution necessitates multiple reentries of memory B cells into GCs to "update" the repertoire and raise robust and high-affinity antibodies against genetically evolving pathogens. Sungalee et al. elegantly demonstrate that this normal physiological process can be subverted by t(14;18)(q32;q21) translocation and ultimately cause FL (25).

The "chronic infection model," which links memory B cell dynamics and follicular lymphomagenesis, has several important implications. First, it provides an excellent example of the initiation of lymphoid malignancies from defects in normal physiological processes, which is exemplified by the memory B cell dynamics demonstrated by Sungalee et al. Second, it dispels the theory that constitutive BCL2 expression is sufficient to allow FL progression. Third, the model supports the increased incidence of disease with age. Fourth, the presence of AID activity in t(14;18)(q32;q21)[+] cells explains FL evolution at a molecular level. Finally, the observations by Sungalee et al. provide proof of principle for previous epidemiological studies suggesting that delayed, recurrent, and chronic infections predispose humans to developing B lymphoid malignancies (37–41).

In light of the findings of Sungalee and colleagues (25), it may be worthwhile to investigate whether these studies can be extended to further examine other B lymphoid malignancies that require additional genetic lesions for transformation. Studies in this direction may unveil a previously unidentified mechanism by which infection leads to clonal evolution in certain subgroups of pediatric pre–B cell leukemias. Such investigations are ongoing for the *ETV6-RUNX1* subgroup of pre–B cell ALL, in which Greaves and Wiemels have proposed that delayed pathogen exposure resulting in chronic and damaging immune responses during early childhood may predispose children carrying this rearrangement to overt leukemia (the so-called "delayed infections hypothesis," refs. 37, 38).

Acknowledgments

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