

IN THE SPOTLIGHT

Infection and the Perils of B-cell Activation

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Summary: Recent studies have linked aberrant B-cell activation in the context of aberrant immune responses to infectious pathogens to malignant transformation and development of leukemia and lymphoma. A new study in this issue demonstrates that common infections can be drivers of clonal evolution of premalignant B-cell precursors toward childhood leukemia. *Cancer Discov*; 5(12); 1244-6. ©2015 AACR.

See related article by Martin-Lorenzo et al., p. 1328 (10).

B cells are unique in their ability to generate antibodies against infectious pathogens and refine their specificity in multiple rounds of mutation and clonal selection (1). Through genetic mutation and recombination events, activated B cells can continuously adapt to antigen. Thereby, B cells undergo a Darwinian selection process that favors clones that have evolved antibodies with the highest affinity to antigen derived from infectious pathogens. Although this process is essential for adaptive immune responses, the same mechanisms of genetic diversification and Darwinian selection can also drive clonal evolution in cancer (ref. 2; Fig. 1). The propensity of B-cell affinity maturation to potentially deleterious genetic lesions led to the concept that repetitive or chronic infections will drive mutation and recombination events of immunoglobulin (Ig) genes in B cells to diversify the antibody repertoire but also increase the risk of malignant transformation. RAG genes mediate V(D)J recombination, and AID drives somatic hypermutation and class-switch recombination of Ig genes. However, both RAG and AID can accidentally act outside of Ig loci and potentially target oncogenes and tumor suppressors. The activity of RAG and AID is typically segregated to early and late stages of B-cell development, respectively. However, the two enzymes can be concurrently expressed under conditions of abnormal B-cell activation by bacterial lipopolysaccharides (LPS) and potentiate the risk of acquiring genetic lesions when acting together (3, 4).

Indeed, infection by *Helicobacter pylori* (*H. pylori*) has been recognized as a critical driver of mucosa-associated lymphoid tissue B-cell lymphoma, which is often reversible by antibiotic therapy. Likewise, chronic B-cell activation in the context of hepatitis C virus (HCV) infection was identified as a causative agent in the etiology of HCV-associated diffuse large B-cell lymphomas. Key evidence for this concept comes from a recent study on the role of *Plasmodium falciparum* (*P. falciparum*) in the etiology of endemic Burkitt lymphoma (5). In a genetic mouse

model for chronic recurrent malaria infection, this study demonstrated that chronic B-cell activation in this model causes aberrant activation of AID and AID-mediated genomic instability leading to B-cell lymphoma. Of note, development of B-cell lymphoma in this model was critically dependent on protracted AID activity (somatic hypermutation and class-switch recombination) in the presence of *plasmodium* infection.

Initial genetic lesions spawning preleukemic clones in acute lymphoblastic leukemia (ALL) usually arise *in utero* (e.g., the common *ETV6-RUNX1* fusion; ref. 6). However, as evidenced by studies with unselected newborn cord bloods and monozygotic twins with ALL, additional genetic changes, including recurrent copy-number changes (mostly deletions), are required for development of clinical ALL, and these are postnatal in origin (6). Epidemiologic evidence supports a “delayed infection” hypothesis predicting that common infections promote those latter secondary genetic events, but only in the context of prior deficits in infectious exposure of infants (7). A meta-analysis of 15 studies indicated that infectious exposures in the first year of life significantly reduce the risk of ALL (8). This cancer has a major peak in incidence in developed societies of 3 to 5 years, concomitant with the period when children are commonly exposed to infections via peer group social contacts. In addition, in newborns, low levels of IL10, a cytokine that limits the duration and intensity of B-cell activation, were recently identified as a predictor of an increased risk to develop leukemia (9). Other data on the impact of certain vaccinations in infancy also accord with a role for infection-associated immune dysregulation in the pathogenesis of childhood ALL (7).

Recent genetic studies in a mouse model provide additional support for this model and implicate cooperative activity of RAG and AID enzymes (3, 4). Mouse B-cell precursors carrying the *ETV6-RUNX1* fusion alone failed to initiate leukemia in transplant recipient mice. However, when *ETV6-RUNX1* B cells underwent repetitive hyperactivation with bacterial LPS, they acquired secondary lesions that enabled development of fatal leukemia in transplant recipients (4). Acquisition of these lesions was dependent on both RAG and AID. Genetic lesions in patient-derived childhood ALL samples revealed a strong bias for known hypermutation target genes of AID. In addition, deletions and gene rearrangements in these patient samples were often targeted to known RAG-specific recombination signal sequence motifs that randomly occur in the genome and showed junctional insertions at break

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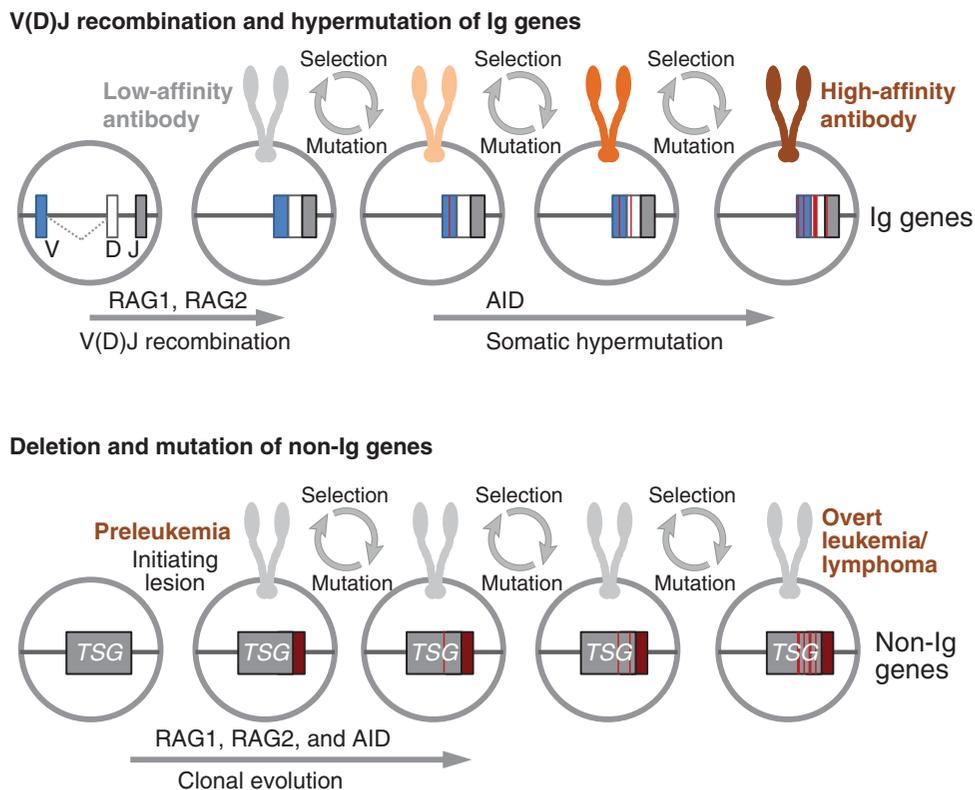


Figure 1. A schematic to illustrate common mechanisms of normal B-cell affinity maturation (top) and clonal evolution toward leukemia and lymphoma (bottom). The activity of RAG1 and RAG2 enzymes in V(D)J recombination and AID in somatic hypermutation of Ig genes is depicted. Multiple rounds of mutation and selection lead to expression of high-affinity antibodies (top). Chronic and repetitive B-cell activation (e.g., through aberrant immune responses to infection) can also lead to accidental targeting of RAG1, RAG2, and AID to non-Ig genes, including tumor suppressor genes (TSG; bottom). Multiple rounds of mutation and selection favor the outgrowth of individual clones based on competitive fitness and drive clonal evolution toward overt leukemia or lymphoma. Recent studies identified *H. pylori*, HCV, and *P. falciparum* as specific infectious pathogens driving of progressive B-cell transformation. Here, Martín-Lorenzo and colleagues (10) demonstrated that common infections can be drivers of clonal evolution of premalignant B-cell precursors toward childhood leukemia.

points that are characteristic of RAG-mediated recombination events (3, 4).

Although previous work focused on RAG and AID as mechanistic drivers of secondary lesions, an elegant new study by Martín-Lorenzo and colleagues in this issue (10) directly demonstrates, in a model system, that common infections can be drivers of clonal evolution of preleukemic B-cell clones. Previous work revealed *PAX5* haploinsufficiency as a predisposing factor in childhood ALL. This study was based on *Pax5*^{+/-} heterozygous mice to model partial loss of *PAX5* function. Importantly, *Pax5*^{+/-} mice did not develop leukemia unless they were exposed to common infectious pathogens. Under pathogen-free conditions, neither wild-type (0/15) nor *Pax5*^{+/-} (0/14) mice acquired leukemia. In the presence of common infectious pathogens, 9 of 41 *Pax5*^{+/-} mice developed fatal B-cell precursor ALL (pre-B ALL), whereas none of 20 wild-type littermates did. Transplantation experiments and whole-exome sequencing demonstrated that leukemia was initiated in a B-cell-autonomous manner. Strikingly, the developing leukemia clones not only recapitulated typical aspects of human pre-B ALL phenotype (surface markers and gene expression pattern) but also acquired the same genetic lesions that were previously identified in human pre-B ALL. These lesions included activating

Jak3 mutations in 6 of 9 mice, 4 of which carried the mouse homolog of the human *JAK3*^{R657Q} mutation. In addition, two mice acquired a mutation of the remaining wild-type *Pax5* allele, resulting in further reduction of *PAX5* activity.

H. pylori, HCV, and *P. falciparum* were identified as specific infectious pathogens that drive progressive B-cell transformation toward lymphoma. The identity of infectious pathogens responsible for the acquisition of postnatal genetic lesions leading toward childhood ALL, however, is still elusive. This current study did not focus on specific subclasses of infectious pathogens that are responsible for clonal evolution of *Pax5*^{+/-} B-cell precursors toward leukemia. However, for future extensions of this work, it would be of great interest to pinpoint the relevant infectious agents, which may help to develop new preventive strategies for childhood ALL. In addition, only 9 of 41 *Pax5*^{+/-} mice exposed to infectious pathogens developed leukemia, which raises the question of whether development of leukemia in this cohort correlated with an abnormal immune response status in the mice that developed leukemia. Testable predictions in this model would be a negative correlation with IL10 serum levels, higher levels of B-cell activation markers, and higher expression and activity of AID and RAG. These new modeling

data from Martín-Lorenzo and colleagues provide strong support for the idea that common infections in childhood can promote the evolution of preleukemic clones toward ALL. Given the ongoing debate about the potential usefulness of government-mandated vaccination programs during early childhood in the United States and elsewhere, further studies building on the work by Martín-Lorenzo and colleagues will be relevant and timely. Together with previous work elucidating the role of *H. pylori*, HCV, and *P. falciparum* as infectious drivers in B-cell lymphoma, the new findings support a broader scenario in which chronic B-cell activation increases propensity to malignant transformation. AID and RAG, mutagenic enzymes that beneficially promote a diverse antibody repertoire, accidentally orchestrate this process.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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