COMMENTARY

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TO THE EDITOR:

Structure and diversification of immunoglobulin genes in African Burkitt lymphoma

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The article by Lombardo and colleagues[1] described high throughput sequencing of the immunoglobulin (Ig) heavy and light chain genes from African Burkitt lymphomas (BLs). On the basis of their analysis and, in particular, because of the frequent lack of detection of Ig D_HJ_H or $V_HD_HJ_H$ rearrangements on the nonexpressed Ig heavy (IgH) chain allele, as well as ongoing mutation on the nonexpressed allele, the authors conclude that BLs derive from an abnormal B-cell progenitor.

I would like to point out several features of the diversification processes of human Ig genes and the pathogenesis of BLs that were not considered in the interpretation of the data and that bring into question the conclusions in the article by Lombardo et al.[1]

First, the article states that all normal B cells undergo biallelic D_HJ_H rearrangement at the pro-B cell stage. This statement is correct for murine B cells but does not hold true for human B-cell progenitors. Although there are few studies on this issue, a detailed Southern blot analysis revealed that about 20% of human B cells lack D_HJ_H or $V_HD_HJ_H$ rearrangements on the nonexpressed IgH allele,[2] so that a considerable fraction of normal human B cells shows a germline configuration of the nonexpressed IgH locus.

Second, it is physiological for human B cells and not an abnormal feature of BL cells that nonproductive $V_HD_HJ_H$ rearrangements undergo somatic hypermutation as efficient as the productive alleles.[3,4] The nonproductive $V_HD_HJ_H$ rearrangements are apparently well transcribed (which is considered an essential prerequisite for being targeted by somatic hypermutation), but because of premature stop codons, the messenger RNA (mRNA) is fast degraded by the process of nonsense-mediated decay and other mechanisms, so that in the steady state, one finds few transcripts from the nonproductive allele.[5,6]

Third, practically all BLs carry translocations of the *MYC* oncogene into 1 of the Ig loci, mostly the IgH locus.[7] These translocations either target 1 of the IgH switch regions or they occur as mistakes of somatic hypermutation and are then found within or close to rearranged Ig V region genes.[4,7] In endemic BL, the *MYC* translocations seem to be frequent in or near rearranged IgH V region genes.[8] If the translocation disrupts the nonexpressed $V_HD_HJ_H$ rearrangements (the translocations do not disrupt the productive allele, because BLs are nearly always surface Ig-positive), then the original rearrangement cannot be further amplified by polymerase chain reaction (PCR).

Fourth, it is to be expected that some $V_HD_HJ_H$ rearrangements were not successfully amplified because of a technical failure of the PCR. The rearrangements were amplified with primers binding to the V_H leader region and to the *IGHJ* gene segments. In both regions, somatic hypermutation occurs, and a single mutation in the Ig V region gene close to the 3′ of the primer binding site will impair efficient amplification of such rearrangements. Thus, when studying mutated B cells, a fraction of mutated Ig V gene rearrangements will likely not be amplified with a strategy using primers in the somatic mutation target sequence. Moreover, about 40% of mutated nonproductive $V_HD_HJ_H$ rearrangements of normal human B cells carry deletions and/or insertions/duplications, which are sometimes several hundred bases long and thus can also prevent successful amplification by PCR.[4] The chance to amplify mutated Ig V genes is higher when using mRNA and C-region–specific primers instead of J gene segment primers because there are no mutations in the C-region exons. But for nonproductive alleles, as pointed out above, one will likely miss them because of the low steady state mRNA levels of such rearrangements.

Taken together, the frequent failure to detect 2 $V_HD_HJ_H$ rearrangements or 1 $V_HD_HJ_H$ and 1 D_H-J_H rearrangement in the endemic BLs can be explained by (1) the physiological germ line configuration of the IgH locus in a fraction of B cells, (2) the destruction of $V_HD_HJ_H$ rearrangements through *MYC* translocation events in germinal-center B cells as a by-product of somatic hypermutation, and (3) occasional failure to

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amplify $V_HD_HJ_H$ rearrangements because of mutations at primer binding sites or larger deletions/duplications. Although exact frequencies for these 3 factors are difficult to estimate, together they can well explain the experimental result in the study on endemic BL. In addition, the detection of somatic mutations in nonproductive $V_HD_HJ_H$ rearrangements is a physiological event and not a marker for an abnormal B cell. Overall, although it cannot be excluded that the B-cell precursors of BL have some peculiarities (eg, they are selected to express particular *IGHV* segments), the features described in the study by Lombardo et al[1] cannot be taken as convincing arguments for a derivation of BL from an abnormal B-cell progenitor and for aberrant mutational processes in these cells.

Finally, a remarkable observation in the study by Lombardo et al[1] is the frequent detection of somatic mutations in D_HJ_H joints. There is very little information on how frequently this occurs in normal or malignant human B cells. For the mouse, it has been reported that the mutation frequency of D_HJ_H joints is only about 10% of that seen in $V_HD_HJ_H$ rearrangements.[9]

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