Brief report

Chronic lymphocytic leukemia B cells expressing AID display dissociation between class switch recombination and somatic hypermutation

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In B cells, somatic hypermutation (SHM) and class switch recombination (CSR) depend on the activation-induced cytidine deaminase (AID) gene product, although the precise mode of action of AID remains unknown. Because some chronic lymphocytic leukemia (CLL) B cells can undergo CSR without SHM, it constitutes a useful model to dissect AID function. In this work, we have studied AID expression, the presence of mutations in the preswitch μ DNA region, CSR, and the SHM in 65 CLL patients. Our results demonstrate that unmutated CLL B cells can constitutively express AID and that AID expression is associated with the presence of mutations in the preswitch region and in clonally related isotype-switched transcripts. They also demonstrate that in CLL without constitutive AID expression, AID induction on stimulation results in preswitch mutations and the CSR process. Our results show a dissociation between SHM and CSR in CLL and suggest that, in this disease, AID would require additional help for carrying out the SHM process. (Blood. 2003; 101:4029-4032)

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termed circle transcripts (CTs), were analyzed by PCR with primers I-γ (forward) and C-μ (reverse), as described by Kinoshita et al.11

Mutation analysis of $V_H$ and preswitch regions

Sequences of $V_H$ genes were determined as previously described.12 Mutations in the Iκ/Sμ region were studied by PCR using Taq High Fidelity polymerase (Roche Diagnostics, Mannheim, Germany) with the following primers: (A) 5'-GGC TGA CCG AAA CTG AAA AGG C-3'; and (D) 5'-GAAAGC TGAGATG ACTG GCC-3'.

Results and discussion

CLL B cells can constitutively express AID transcripts, which predominate among unmutated cases and are associated with the expression of additional clonally related transcripts

Unmutated cases predominated among aggressive forms of CLL (25 of 34, stages B and C) whereas mutated cases predominated in stage A (23 of 31). By using a stringent semiquantitative reverse transcription (RT)-PCR, we could substantiate constitutive AID expression in 10 of 65 patients, which might have accounted for a lower incidence when compared with another series11 (Figure 1A). Interestingly, all these patients expressed unmutated rearranged $V_H$ genes and displayed either advanced disease (stage B in 4) or progressive disease (stage A in 6). As shown in Figure 1B, different AID transcripts were amplified by RT-PCR, corresponding, respectively, to the complete sequence of AID transcript previously reported by Revy et al.,2 a fragment displaying a 10-amino acid deletion in the initial portion of exon 4, and a fragment with a 51-amino acid deletion including exons 4 and 5. Different AID-splicing variants have also been reported by Noguchi et al.14

The expression of constitutive AID transcripts in 10 unmutated patients led us to examine the CSR process by analysis of clonal isotype switch transcripts and the presence of γ-CTs as recently reported in CLL by Cerutti et al.15 Seven of these 10 CLLs expressed μ, δ, γ, and α transcripts (Figure 1C), which were substantiated to display $V_H$ sequences identical to those expressed by the tumoral clone (data not shown). In addition, γ-CTs were observed in all these unmutated patients, indicating that the initiation of a CSR process11 is accounted for in these B-CLLs (data not shown).

AID expression enables CLL-B cells to carry out CSR somatic-like mutations but not SHM

To further investigate the dissociation between SHM and CSR in B-CLLs cells, taking into account that upon stimulation in the conditions that induce CSR, AID induction is associated with hypermutation in the Sμ region under the conditions that induce CSR.16 We have studied B cells from 4 healthy donors and 7 patients with CLL (4 unmutated and 3 mutated) before and after

Figure 1. Expression of AID RNA transcripts in CLL and their relation in CSR and SHM process. (A) Semiquantitative AID expression. The expression of AID transcripts was monitored by semiquantitative RT-PCR using AID and GAPDH-specific primers in the same RT-PCR tube reaction. Representative amplification for healthy B cells (00) and either unmutated (01 and 03) or mutated (05 and 07) B-CLLs are shown. Amounts of AID transcripts were determined by normalization with internal GAPDH expression. Relative units corresponding to AID and GAPDH transcript amplification levels were quantified by Quanti software (Molecular Dynamics). (B) Presence of different AID RNA transcripts. Normal cDNA (00) and CLL cDNA (01) were amplified and migrated. Three different RNA forms of the AID gene were found (1, 2, 3). The figure depicts a schematic sequence of AID mRNA previously reported by Schroeder et al.11 corresponding to 198 amino acids. The other 2 variants are spliced forms, one consisting of 618 base pair (bp) with an open-reading frame containing a deletion of 10 amino acids and the other consisting of 495 bp containing a complete deletion of exons 4 and 5 (51 amino acids). Deletions are depicted as unfilled rectangles. (C) Clonal isotype switch transcripts. mRNA transcript amplifications with tumor-related $V_H$ primers in 5' and Cμ, Cδ, Cγ, and Cα in 3' from patients 1 and 3 with unmutated and patients 5 and 7 with mutated disease. Patient 1 expresses μ, δ, γ, and α transcripts, and patient 3 expresses μ, δ, and γ transcripts related to the tumoral clone. The smearlike amplification observed for patient 7 corresponds to a polyclonal amplification of different γ transcripts as confirmed by sequence. After stimulation patient 5 expressed a tumoral related γ transcript, and patient 7 acquired tumoral related γ and α transcripts. (D) Distributions of mutations in the Iκ/Sμ region. A 1625-bp genomic fragment between the enhancer and the Sμ switch core was amplified with primers A and D for 4 healthy controls and 7 patients with CLL (Table 1). Closed and open arrows indicate point mutations (30 in the 3' subregion, 9 in the 5' subregion). In addition, 4 deletions in the 3' subregion, depicted as rectangles, were observed. Open arrows illustrate where repeated mutations took place. Primers B (5'GAG CTG CTG GTC TGC TGC AAG AGG CAG CAG CGG-3') and C (5'GAC ATG GTA AGA GAC AGG CAG CGG-3') were used as internal primers for sequencing reaction. Given that in all cases 3 independent sequences obtained from different PCRs with a high-fidelity Taq DNA polymerase (10−5 expected rate mutation) were carried out, Taq fidelity should play a minor, if any, role in the appearance of mutations.
Table 1. Implication of AID expression in mutational profile of Iκ/Sm region, CSR, and SHM

<table>
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<tr>
<th>Sample DNA and RNA stimulation, CD40L + IL-4 for 5 days</th>
<th>AID transcripts, RT-PCR</th>
<th>SHM homology to germinome, %</th>
<th>5' region Mutations</th>
<th>Deletions</th>
<th>3' region Mutations</th>
<th>Deletions</th>
<th>Complete region Mutations/bp sequences</th>
<th>Clonal isotype switched transcripts, RT-PCR</th>
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Results of 3 independent colony experiments are shown together. N/C indicates no clonal VDJ gene.

Acknowledgments

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References