To the editor:

Immunoglobulin *(Ig)/BCL6* versus non-*Ig/BCL6* gene fusion in diffuse large B-cell lymphoma corresponds to a high- versus low-level expression of *BCL6* mRNA

We read with interest the manuscript of Lossos et al,[1] in which they disclosed the impact of *BCL6* mRNA expression on the clinical outcome of diffuse large B-cell lymphoma (DLBCL). The authors established a quantitative real-time polymerase chain reaction (PCR) method using TaqMan technology to measure the levels of *BCL6* mRNA in clinical materials. They first analyzed 22 cases of DLBCL and found that the overall survival (OS) of patients with high *BCL6* expression was significantly superior to that of patients with low *BCL6* expression, and their finding was validated in an additional 39 cases. They suggested that high *BCL6* mRNA expression is a new favorable prognostic factor for DLBCL.

3q27 translocation affecting *BCL6* gene has been observed in 20% to 40% of DLBCL.[2] The particular feature of *BCL6* translocation is that it can involve not only either one of the 3 immunoglobulin genes (*Ig*) but also another non-*Ig* partner.[2,3] Our initial study suggested that DLBCL cases with non-*Ig*/*BCL6* translocation showed a worse prognosis than those with *Ig/BCL6*.[4] In an updated comparison between 17 cases with non-*Ig* partners, including 2 with a deletion of a larger than 1-kb segment encompassing the *BCL6* exon 1, and 26 cases with *Ig* partners, OS of the former group was inferior to that of the latter group ($P = .0400$). Thus, we propose that the non-*Ig/BCL6* fusion gene is a poor prognostic indicator of DLBCL.

To test the correlation between the 2 independent studies on the prognostic significance of *BCL6*, we compared the levels of *BCL6* mRNA between DLBCL cases with *Ig/BCL6* translocation and those with non-*Ig/BCL6*. Total cellular RNA were prepared from cryopreserved tumor cells and subjected to real-time PCR analysis using an ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA). The sequences of oligonucleotide primers as well as the fluorogenically labeled probe were as described.[1] The amount of *BCL6* mRNA of test materials was divided by that of the endogenous reference, the glyceraldehydes-3-phosphate dehydrogenase gene (*GAPDH*), and the *BCL6-GAPDH* values were further normalized with that of Raji cells.[1] As indicated in Figure 1, the comparison study clearly showed that *BCL6* mRNA levels of the *Ig/BCL6* group (n = 6; range, 2.2-7.0; mean, 4.3) were significantly higher than those of the non-*Ig/BCL6* group (n = 8; range, 0.4-1.9; mean, 1.0) ($P = .0003$). In contrast, the values of DLBCL cases that lacked *BCL6* rearrangement were distributed from 1.2 to 10.7 (n = 9; mean, 6.5). We next performed reverse transcriptase–mediated PCR using a forward primer for *BCL6* exon 1 and a reverse primer for exon 4. There were no measurable amounts of normal *BCL6* transcripts in cases with *BCL6* translocation, though additional PCR using nested primer sets generated PCR products in a proportion of the materials tested. It is therefore likely that the observed *BCL6* mRNA in both *Ig/BCL6* and non-*Ig/BCL6* cases represented transcription not of the normal *BCL6* but of the rearranged *BCL6* allele.

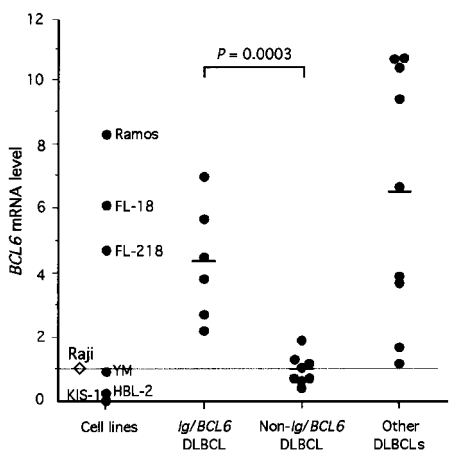

Figure 1. *BCL6* mRNA expression levels in lymphoma cells determined by real-time PCR. The *BCL6/GAPDH* ratio of each test material was normalized by that of Raji (= 1). Cell lines: Ramos, a Burkitt lymphoma cell line with t(8;14)(q24;q32); FL-18 and FL-218, follicular lymphoma cell lines with t(14;18)(q32;q21); YM, a DLBCL cell line with t(3;16)(q27;p11)[6]; HBL-2 and KIS-1, DLBCL cell lines with t(11;14)(q13;q32) and t(9;14)(p13;q32), respectively. Clinical materials of DLBCL: DLBCL with *Ig/BCL6* translocation, n = 6; DLBCL with non-*Ig/BCL6* translocation, n = 8; other DLBCLs lacking *BCL6* translocation, n = 9. Horizontal bars indicate the mean values of each group.

DLBCL is a heterogeneous subcategory of non-Hodgkin lymphoma in terms of cell morphology, immunophenotype, and genetic abnormality. Many studies have focused on whether these parameters are associated with particular clinical features and the treatment outcome of DLBCL.[5] Lossos et al[1] and our own studies,[4] including this report, suggest that *Ig* versus non-*Ig* partner of *BCL6* translocation and high versus low *BCL6* mRNA expression are both prognostic indicators of DLBCL, potentially reflecting a role of *BCL6* in the pathogenesis of DLBCL. We very recently found that t(3;16)(q27;p11) translocation leads to the fusion of *BCL6* with the interleukin-21 receptor gene *(IL-21R)*.[6] Although *IL-21R* was actively transcribed in a DLBCL cell line (YM) carrying this particular non-*Ig/BCL6* translocation, the level of *BCL6* mRNA, which was under the control of *IL-21R* promoter, was unexpectedly low (Figure 1). Thus, it is possible that *BCL6* expression is down-regulated in lymphoma cells with t(3;16) once the cells have obtained a malignant phenotype. In contrast, lymphoma cells with *Ig/BCL6* may show a persistent expression of *BCL6* at higher levels, corresponding to a feature of germinal center B-like DLBCL.[7] Further studies are needed to elucidate the mechanistic detail involved in the transcriptional control of *BCL6* in lymphoma cells.

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To the editor:

Enhanced clearance of leukemic lymphocytes in B-cell chronic lymphocytic leukemia with etodolac

Chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by the accumulation of small mature-appearing lymphocytes in the blood, bone marrow, and lymphoid tissues.[1] Current therapy has not been shown to prolong survival.[2]

Etodolac is a racemic mixture of R-(-) and S-(+)-1,8-diethyl-1,3,4,9,-tetrahydropyrano-(3,4-b)indole-1-acetic acid.[3] Etodolac selectively inhibits cyclooxygenase-2,[4] and it is approved for use in various parts of the world for treatment of degenerative joint disease and rheumatoid arthritis and for use as an analgesic.[5]

We report the chance observation that racemic etodolac lowers the lymphocyte count in a patient with B-CLL and show that challenges with 13 other nonsteroidal anti-inflammatory drugs (NSAIDs) produced no significant effect. We also present studies to show that at standard anti-inflammatory doses, racemic etodolac achieves this action by enhancing the selective clearance rather than by the direct killing of leukemic lymphocytes.

The patient's condition was diagnosed as B-CLL in 1994 at age 57. Her leukemic lymphocyte phenotype was $CD5^+$, $CD19^+$, $CD20^+$, $CD23^+$, $CD25^+$, $FMC7^+$, and λ dim^+. She was clinically staged at Rai/Binet 0/A. On October 23, 1997, her white blood cell count (WBC) was 34 300 and her lymphocyte count was 27 440. From October 25, 1997, to October 27, 1997, she took etodolac 300 mg PO twice a day for neck pain. On October 28, 1997, because of the development of petechiae etodolac was discontinued and a complete blood count was performed. Her platelet count was normal but her WBC had dropped to 13 400 with a lymphocyte count of 6 700. To determine whether this was a reproducible effect, etodolac at the same dose of 300 mg was administered twice a day from November 5, 1997, to November 11, 1997; January 21, 1998, to January 25, 1998; February 26, 1998, to March 2, 1998; and April 8, 1998, to April 13, 1998, all with similar effects (Figure 1A). No clinically significant changes in the hemoglobin level, neutrophil count, or platelet count were seen with etodolac (platelet data not shown). Also seen in Figure 1A and B are the results of the administration of 13 other NSAIDs. None produced any significant decrease in the lymphocyte count. The effect of etodolac appears not to be due to its anti-inflammatory property since none of the other NSAIDs elicited this response. Furthermore, the effect does not appear to be related to cyclooxygenase specificity since celecoxib, rofecoxib, and meloxicam, selective cylooxygenase-2 inhibitors, did not significantly affect the lymphocyte count. A 4-month course of etodolac was then administered between July 20, 1998, and November 23, 1998 (Figure 1C). Taken together with the previous data, the reduction of lymphocyte count in response to etodolac administration was prompt, with a nadir occurring after 2 days of administration. The duration of the effect after drug withdrawal was short, with a return of lymphocyte count to baseline within 2 weeks of drug cessation independent of the duration of therapy. The effect was also reproducible and did not appear to be lessened with repeated or prolonged drug challenges.

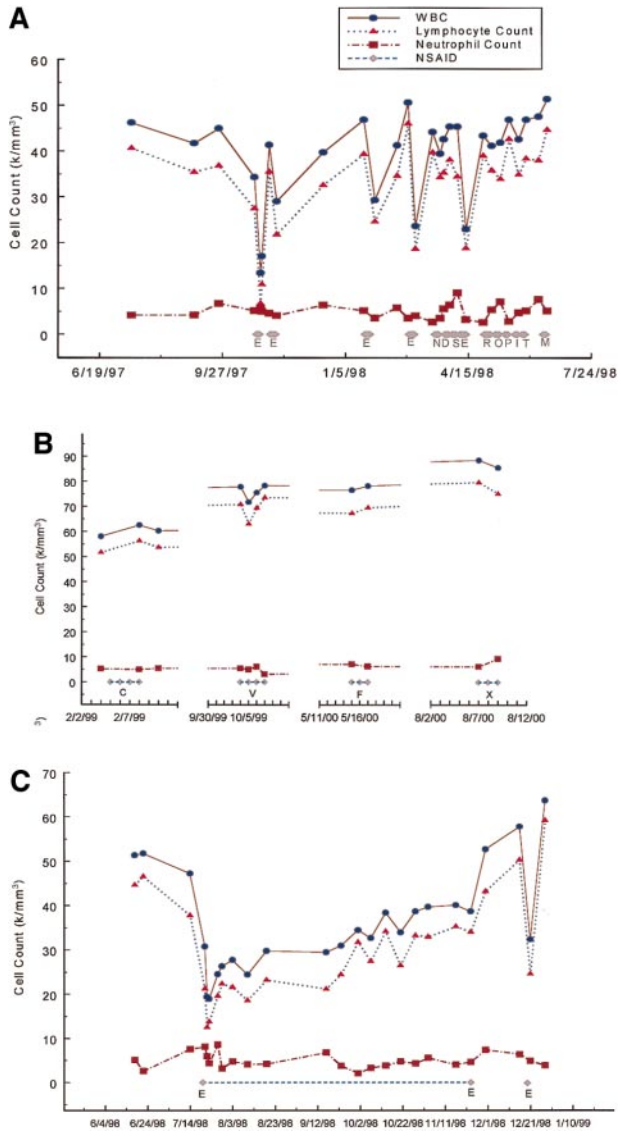

Figure 1. Cell counts as a function of NSAID administration. (A) Effects of E, etodolac, 300 mg twice a day for 3 to 6 days, compared with 9 other NSAIDs: N, naproxen, 250 mg twice a day for 4 days; D, diclofenac, 50 mg 3 times a day for 4 days; S, sulindac, 200 mg twice a day for 4 days; R, nabumetone 500 mg twice a day for 6 days; O, oxaprozin, 600 mg twice a day for 4 days; P, piroxicam, 20 mg every day for 3 days; I, indomethacin, 25 mg 3 times a day for 3 days; T, tolmetin, 400 mg twice a day for 3 days; and M, ibuprofen, 400 mg 3 times a day for 3 days. (B) Effects of C, celecoxib, 100 mg twice a day for 4 days; V, rofecoxib, 25 mg every day for 4 days; F, flurbiprofen, 100 mg 3 times a day for 3 days; and X, meloxicam, 7.5 mg every day for 3 days. (C) Effect of a 4-month course of etodolac. A 400 mg sustained release preparation was administered twice a day from July 20, 1998 until July 28, 1998, followed by 300 mg twice a day of the standard release preparation until September 8, 1998, followed by reinstitution of the 400 mg sustained release preparation twice a day. There was a rapid decline in the lymphocyte count in the first several days to a nadir of 12 510 followed by rebound and gradual increase to 33 968 on November 23, 1998, the last day of etodolac administration. After discontinuation of etodolac, there was a rapid rise of the lymphocyte count to 43 132 on November 30, 1998, and to 50 199 on December 16, 1998. To determine if a full response to etodolac was still possible after a prolonged course, etodolac was administered at 400 mg sustained release twice per day for 2 days from December 19, 1998, to December 21, 1998. The lymphocyte count on December 21, 1998, dropped to 24 548 with a rapid return to 59 148 by December 28, 1998.