The Juxtaposition of ABL With BCR and Risk for Fusion May Come at the Time of BCR Replication in Late S-Phase

To the Editor:

In their recent report in Blood, Neves et al. demonstrated the close proximity of BCR and ABL in hematopoietic cells in late S-phase of the cell cycle and provided a basis for a model to explain the t(9;22) chromosomal abnormality. We have studied the relative positioning of BCR and ABL in cells that happened to be accumulating in late S- and G2M-phase, and observed a similar proximity of the 2 genes. In our evaluation, the juxtaposition appears to occur at the time of the replication of the second of the 2 BCR loci.

The cells analyzed were from a 49-year-old man who had just begun reinduction for a relapsed acute leukemia. The patient was initially diagnosed morphologically as having acute myelogenous leukemia a year earlier at an outside hospital, but on transfer to the University of Chicago was found to have circulating blasts (white blood cell [WBC] count, 4.9 × 10⁹/L, 8% blasts) with a common precursor B-cell phenotype (CD19⁺, CD10⁻, TdT−, CD34⁺, slg−, CD13−, and CD33−, MyPs−). Fluorescence in situ hybridization (FISH) analysis with probes for MBCR and ABL (Oncor, Gaithersburg, MD) was performed on a peripheral blood buffy coat smear (2% blasts) to determine if the lymphoblasts were BCR/ABL⁺, and if so whether other cell types were also positive. Inadvertently, the specimen for FISH was obtained 15 hours after the onset of therapy with cyclophosphamide, daunorubicin, vincristine, and prednisone.

The FISH results, when correlated with the morphology of the previously Wright-stained cells, showed no consistent BCR/ABL fusion signals in the blasts or other cell types. This was supported by cytogenetic and molecular analyses of an involved bone marrow, which showed that the patient had a normal karyotype, and no BCR/ABL fusion transcripts for p190 or p210 by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. The FISH results did show, however, that most of the blasts (16 of 27 evaluated) appeared to be in late S- or G2M-phase, with many cells showing 3 or 4 copies of both ABL and BCR (Fig 1A through C). This cell-cycle distribution presumably was an effect of the recently administered vincristine. The analysis also showed that the BCR and ABL signals tended to approach one another when there were 6 or 7 total signals (consistent with late S-phase) (Fig 1A), to merge at a time when the second BCR seemed to be replicating (Fig 1B, and inset), and to separate at G2M (Fig 1C). The distance between the 2 closest BCR and ABL signals was shortest (approaching 1 signal diameter length) when there were 6 or 7 total signals, and this was statistically different from the shortest separation in cells with 4/5 or 8 total signals (P < .0001 and P < .001, respectively).

Our chance evaluation of cells from a patient who had already begun therapy permitted us to observe BCR and ABL positioning in the otherwise infrequent cells of late S and G2M. Although the small number of cells studied provide somewhat limited data, our finding supports the results of Neves et al, showing close juxtaposition of the 2 genes in late S-phase of primitive hematopoietic cells. Our finding further advances their model by suggesting that the actual juxtaposition of the 2 genes may occur around the time of the replication of BCR. Our illustrations clearly show an approach, convergence, and separation of the 2 genes when there is full tetraploid complement of ABL signals, and when BCR copy number goes from 3 to 4. Although the close proximity of BCR and ABL was identified in late S-phase cells in the work of Neves et al, the replication status or copy number of the BCR and ABL genes is not apparent from their data. In fact, it is curious that in their Fig 1, the late S-phase cell depicted seems to have only a diploid complement (2 signals each) of BCR and ABL.

Our finding of BCR and ABL closest juxtaposition in late S-phase at a time near an apparent BCR replication would support the possibility of the BCR/ABL fusion developing during a replication error in BCR. Recombination may more likely occur during DNA replication, and this...
Replication of Hepatitis C Virus in B Lymphocytes (CD19+)

To the Editor:

In recent years, the presence of hepatitis C virus (HCV) genomic sequences (plus-strand) and replicative intermediate (minus-strand) in the peripheral blood mononuclear cells (PBMC) has been reported. To clarify which PBMC subpopulation is infected, and to evaluate active production of viral particles in target cells, we searched for the presence of viral genomic and antigenomic-RNA in PBMC and PBMC subset of 4 HCV chronically infected patients, before and after mitogenic stimulations of cells.

Peripheral blood mononuclear cell subpopulations (CD4+, CD8+, CD19+) were used to eliminate cross-contamination of cell subset with other cells, and confirm that the detection of HCV-RNA in purified PBMC subpopulations was caused by active replication rather than by passive adsorption of virions or contamination with serum-associated viral particles.

Purification of cells was performed by microbeads separation (Miltenyi Biotec, Bergisch Gladbach, Germany) at standard condition; after flow-cytometric analysis by FACScan (Becton Dickinson, Milan, Italy), the purity of sorted cells was provided to be 95% to 97%. Mitogenic proliferation of cultured PBMC and subset populations was evaluated by [3H] thymidine incorporation. Then, HCV-RNA was detected in serum and cells by reverse transcription-nested-polymerase-chain reaction (RT-n-PCR) for the highly conserved 5' untranslated region of HCV genome. Minus-strand RNA was detected by using a sense primer instead of an antisense primer for RT. To verify the homogeneity of total RNA extraction from different cell subpopulations, a 10-fold sample dilution of purified cells was amplified for a β-actin gene, showing comparable positive results. HCV-RNA was found to be present in fresh PBMC and resting B lymphocytes of 3 patients, whereas CD4+ and CD8+ resting cells were HCV-RNA positive in only 1 of these 3 patients. HCV genomic sequences were undetectable in fresh total PBMC and subpopulations of 1 other patient. The presence of HCV-RNA was then searched for in total PBMC, CD4+, and CD8+ cells at days 7 and 14, and in B lymphocytes at days 7 and 9 of mitogenic stimulation. In 3 of 4 patients, B lymphocytes were HCV-RNA positive at day 7 of stimulation whereas HCV-RNA was found to be negative in cultured CD4+ and CD8+ subsets of all the patients (Fig 1).

To further confirm our data, the negative-strand RNA, which is a replicative intermediate of HCV, was searched for in serum, total PBMC, and in the subset of cells that were previously found to be plus-strand RNA positive. All serum samples were found to be minus-strand RNA negative. The antigenomic-RNA was detected after n-PCR in the PBMC and B-lymphocyte subpopulation of 1 patient, both in unstimulated cells and after 1 week of mitogenic stimulation (Fig 2).

Finally, HCV genomic sequences were sought in culture supernatants, and were undetectable in culture media of all the cultured cells, suggesting that viral particles are not being released. We found constantly the presence of HCV-RNA in resting and stimulated (with pokeweed mitogen at final concentration of 2.5 μg/mL) B lymphocytes, whereas CD4+ and CD8+ subpopulations were invariably negative after stimulation. In the present study, the minus-strand RNA, which is described to represent an intermediate replicative form of HCV, was detected in fresh and cultured PBMC and B lymphocytes of 1 patient, whereas antigenomic sequences were not detected in the B lymphocyte subset of 2 other patients. It is possible that these patients had a small quantity of minus-strand RNA, which is known to be in a lower concentration compared with positive-strand RNA, or HCV was present but dormant, also after stimulation of cells. Recently, Pileri et al showed that the HCV E2 envelope protein binds to CD81, a tetraspanning expressed in various cells including hepatocytes and B lymphocytes. In particular, this study showed that HCV binds to human B-cell lines. In agreement with these data, we invariably found HCV-RNA in quiescent as stimulated B lymphocytes. In conclusion, we showed the presence of HCV plus and minus-strand RNA in cultured total PBMC and B cells, and these findings strongly suggest that PBMC and, in particular B lymphocyte subsets, may represent extrahepatic sites of HCV replication. Further studies on in vivo and in vitro replication of HCV in PBMC subsets could help to elucidate the viral life cycle in PBMC.

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REFERENCES
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