Abstract. Background: Chronic myelogenous leukemia (CML) is characterized by an initial chronic phase that invariably evolves to the more aggressive phase of blast crisis. Although the determinants of this transition are still unknown, it has been shown that the blast crisis is accompanied by genetic instability.

Materials and Methods: The expression and activity of the error-prone DNA polymerase beta (pol β) were investigated in blood samples from CML patients, by Western blotting and by an in vitro replication assay, respectively. Results: Pol β expression and activity were significantly higher in CML samples compared to those of healthy donors. Conclusion: Our results suggest that the excess of pol β in CML could contribute to the genetic instability observed during the evolution of the disease from the chronic phase to blast crisis.

Chronic myelogenous leukemia (CML) is a fatal disease characterized by the expansion of hematopoietic progenitor cells. The cytogenetic hallmark of CML is the translocation (19;22) which produces a shortened chromosome 22, called the Philadelphia (Ph1) chromosome. This translocation produces the BCR-ABL oncogene, which encodes for a fusion protein with constitutively-activated tyrosine kinase activity. The CML pathology is characterized by a chronic phase followed by an acute phase, called the blast crisis.

During the chronic phase, the Ph1 chromosome remains the major DNA aberration (1), while the blast crisis is characterized by the accumulation of additional molecular changes, including chromosomal abnormalities (2) and mutations in proto-oncogenes and tumor suppressor genes (reviewed in (3)). It has been shown that the BCR-ABL oncogene induces genetic instability both in vitro (4) and in vivo (5). However, the role of BCR-ABL in the generation of this genetic instability is unknown. Previously, we showed that the transfection of the murine Ba/F3 cell line with BCR-ABL resulted in the overexpression of the error-prone DNA polymerase β (pol β), concomitantly with an increased nucleotidic instability (4). Pol β is an essential enzyme involved, at the basal level, in the base excision repair pathway (6). However, when up-regulated, pol β promotes genetic disorders, such as mutagenesis (7), chromosomal abnormalities (8) and aneuploidy (9).

In order to determine whether pol β could play a role in the genetic instability observed in CML, the pol β expression and activity were examined in a panel of sample cells from patients and were compared to samples from healthy donors (HD).

Materials and Methods

Clinical samples. Peripheral blood samples from 5 HD and 9 CML patients at diagnosis were obtained after informed consent from the Department of Hematology, Purpan Hospital (Toulouse, France). The diagnosis of CML was based on standard clinical, hematological and cytogenetic criteria. All patients displayed the Ph1 chromosome and were in the chronic phase.

Cell extract preparation. Neutrophils were isolated from the blood of HD and CML patients, as previously described (10), and resuspended in PBS. The final suspensions contained ~95% neutrophils. Cell extracts were prepared as previously described (4).

Western blot analysis. For the analysis of pol β expression, cell extracts (50 µg protein) were separated in a 10% SDS-PAGE and transferred to a PVDF membrane (Amersham Biosciences, Orsay, France). The blots were blocked in TBS containing 0.1% Tween, with milk (5% skimmed milk) and incubated with pol β monoclonal antibody (clone 18S) (Interchim, Montluçon, France), followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG, and revealed
using the ECL system (Amersham Biosciences). The quantification analysis was achieved by PhosphoImager Storm-system analysis using Imagequant software.

Pol β activity assay. A 60-mer oligonucleotide was hybridized to a 5′-32P-labelled 17-mer synthetic primer to serve as a DNA template. This template was replicated in vitro by the cell extracts, as previously described (4). Pol β activity was expressed as the percentage of the inhibition of DNA synthesis by ddCTP of all products elongated from the primer.

Results and Discussion

Pol β expression was first examined by Western blot analysis. The relative pol β expression was higher in CML patients than in the HD in 7 out of 9 samples (Figure 1). Pol β expression in the HD showed only slight variations in contrast to the samples from the CML patients. Pol β expression was more than 2-fold higher in 4 out of 9 CML samples compared to the control samples. Pol β activity was subsequently measured by monitoring its ability to incorporate the chain terminator ddCTP into DNA (4). In the presence of ddCTP, pol β activity was inversely correlated to the extent of primer extension. Using this assay, the pol β activity in cell extracts from the CML patients at diagnosis were compared to those in cell extracts from HD. As a representative example, the ability of the cell extract from a healthy donor (HD3) and a CML patient (CML5) to incorporate ddC during DNA synthesis is displayed in Figure 2A. In the presence of ddCTP, DNA synthesis was more inhibited in the cell extract from the CML patient than from the HD cell extract. Seven out of 9 of the CML samples at diagnosis were compared with the 4 HD blood samples. The mean value of pol β activity in the CML patients was 2.2-fold higher than that in the HD group (Figure 2B).

Pol β is a key enzyme in the base excision repair mechanism. This DNA pathway is necessary for maintaining cellular genetic stability (6). However, when overexpressed, pol β favors genetic instability by increasing the mutation rate and drug resistance (7). In addition, the overexpression of pol β could alter the cell cycle and induce aneuploidy and tumorigenesis (9), thereby demonstrating the necessity for cells to accurately control the levels of pol β expression and activity. High levels of pol β have been observed in many cancer cells, such as ovarian (11), prostate, breast and colon cancers, compared with levels in adjacent normal tissues (12). As shown in a previous work using the murine BaF3 cell line, the expression of BCR-ABL increased pol β expression. In the present study, we showed, for the first time, that CML cells from patients display enhanced pol β expression and activity. Since the blast crisis transition is associated with cytogenetic and molecular genetic abnormalities, which can both result in overexpression of pol β, the involvement of pol β overexpression in the evolution of CML could be suspected. Taken together, these results show that pol β expression is linked to the expression of the BCR-ABL oncogene and could be one of the determinants of the genetic instability observed during the evolution of CML.

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References


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Figure 2. (A) DNA polymerase β activity in a blood sample from a healthy donor (HD3) and a CML patient (CML5). Pol β activity was measured by monitoring the effect of ddCTP on in vitro DNA synthesis by replicative extracts in blood samples from the CML patient and the HD. Quantification is the mean of 2 separate experiments (+/− SE). (B) Distribution of the pol β activity in blood samples from CML patients and HD. Each point is the mean of 2 separate experiments. Bars represent the mean of pol β activity of the population. The mean value was 15 for the HD group and 34 for the CML patient group.