Differences in the Expression of Alkaline Phosphatase mRNA in Chronic Myelogenous Leukemia and Paroxysmal Nocturnal Hemoglobinuria Polymorphonuclear Leukocytes

By A. Rambaldi, M. Terao, S. Bettoni, R. Bassan, R. Battista, T. Barbui, and E. Garattini

Paroxysmal nocturnal hemoglobinuria (PNH) and the stable phase of chronic myelogenous leukemia (CML) are the two hematological conditions known to be associated with low levels of leukocyte alkaline phosphatase (LAP) activity in peripheral blood polymorphonuclear cells (PMN). LAP mRNA levels were determined in PMN from PNH and CML patients by RNA blotting analysis. In CML, LAP mRNA is undetectable, suggesting either decreased transcription or rapid degradation of the message. Contrarily, in PNH normal or high levels of LAP mRNA are present. This latter finding supports the concept of a deficit in the anchorage of the protein to the plasma membrane through the glycolipid pathway, even though other post-transcriptional mechanisms could be involved.

**Mammalian** alkaline phosphatases (orthophosphoric monoester phosphohydrolases [alkaline optimum EC 3.1.3.1]) constitute a family of closely related membrane-bound glycoproteins. The alkaline phosphatases are one of a growing number of proteins that have been shown to be anchored to the plasma membrane by a phosphatidylinositol (PI) tail.1

Paroxysmal nocturnal hemoglobinuria (PNH) and chronic myelogenous leukemia (CML) in its stable phase are the only two hematological conditions associated with a dramatic and consistent decrease in PMN leukocyte alkaline phosphatase (LAP) activity.2,3 The molecular mechanisms underlying this phenomenon are not yet known. However, in PNH the decreased enzyme activity is probably due to an inefficient anchoring of LAP as well as other PI tail proteins to the plasma membrane.4,5 In CML, it is suggested that the defect in LAP activity may be due to a specific lack of gene transcription.2,6,7

The alkaline phosphatase isoenzyme normally expressed in PMN is also found in liver bone and kidney, and is referred to as the L/B/K form.8 The availability of the cDNA encoding the L/B/K isoenzyme9 prompted us to investigate, by RNA blotting analysis, the steady-state levels of LAP mRNA to elucidate whether transcriptional or post-transcriptional mechanisms are causing the depression of LAP activity in CML and PNH.

**MATERIALS AND METHODS**

**Cell preparation.** Blood samples were obtained from patients and healthy volunteers after informed consent. Six cases of Philadelphia-positive CML in the stable phase of the disease, and two cases of PNH were analyzed. Peripheral blood mononuclear cells (PBMC) and PMN were purified as already described.10 The purity of PMN was greater than 98%. These cell preparations were used for LAP determination and RNA extraction.

**Preparation of total cellular RNA and RNA blotting analysis.** Total cellular RNA was isolated as previously described11 by lysing cells in guanidium isothiocyanate followed by recovery of RNA by centrifugation through cesium chloride.12 Ten μg samples were fractionated on a 1.2% agarose gel with 6% formaldehyde, and blotted onto synthetic nylon membranes (Gene Screen Plus, New England Nuclear, Boston). LAP mRNA was detected by using p-nitrophenol phosphate (Sigma, St. Louis) as substrate after n-butanol extraction.13 LAP enzyme assay was performed as modified by Hayhoe and Quaglino.14

**RESULTS**

**LAP enzyme activity in PNH and CML.** Table 1 shows the relevant features of two typical CML and the two PNH patients analyzed in this report. Their LAP score is compared to LAP scores obtained from our reference population. LAP scores from both PNH and CML patients range from 9 to 16, and, as expected, represent subnormal values. Microscopic observation of the peripheral blood smears revealed that the percentage of LAP positive cells v LAP negative cells was no more than 5% to 10% in PNH samples, and the same was true for CML.

LAP enzymatic activity was also determined in total cell extracts of purified PMN. PNH leukocyte extracts contain 1.3% to 3.5% whereas CML contain 3.5% to 4.0% of the activity found in normal leukocytes (Table 1). Thus, LAP scores and enzymatic activity are in agreement and demon-
The lack of LAP transcript is not due to RNA degradation in analyzed (two typical examples are shown in lanes f and g). CML, no detectable signal is observed in the six cases controls and values of 1.28 and 3.80 for the two patients. In analysis of the autoradiography signals and calculation of the obtained from normal healthy subjects, PNH and CML detectable. The results of RNA blotting analysis on PMN 90% of their PMNs.

strate that the patients chosen to represent the two diseases all have highly depressed levels of LAP activity in more than 90% of their PMNs.

LAP mRNA in PNH is high while in CML is not detectable. The results of RNA blotting analysis on PMN obtained from normal healthy subjects, PNH and CML patients are presented in Fig 1. In both cases of PNH, the levels of LAP transcript are similar to the control levels (compare lanes b and c to lanes a and d). Densitometric analysis of the autoradiography signals and calculation of the ratio LAP-actin RNA gave values of 0.67 and 1.33 for the controls and values of 1.28 and 3.80 for the two patients. In CML, no detectable signal is observed in the six cases analyzed (two typical examples are shown in lanes f and g). The lack of LAP transcript is not due to RNA degradation in this pathological situation since equivalent levels of actin transcript are observed in both control and CML RNA (lanes e, f and g, h).

**DISCUSSION**

The data presented in this report show that two hematological conditions known to be associated with low levels of LAP enzymatic activity in the PMN, PNH and CML, have different steady-state levels of LAP mRNA. PNH PMN express normal or high levels of specific transcript, whereas CML PMN during the stable phase of the disease are characterized by a virtual absence of LAP mRNA.

At least in the case of CML, the decreased levels of LAP activity do not seem to be explained by aberrant structure of the gene. In fact, induction of LAP activity is observed in vivo in various situations and can be produced in vitro by treatment of neutrophils with conditioned medium from cell lines and tumors expressing CSF activity. Recently, we also found that LAP mRNA transcription can be induced by treatment with pure recombinant G-CSF (data not shown).

In CML the lack of detectable LAP mRNA completely explains the highly reduced levels of enzymatic activity in the cases studied. It is yet to be formally established whether the absence of LAP mRNA is due to decreased transcription or to shortened half-life of the transcript.

On the other hand, the presence of LAP mRNA in PNH PMN is consistent with recent observations reported on this disease. Previous studies on cells from PNH patients have demonstrated a selective deficiency of PI tail proteins, including decay accelerating factor (DAF), acetyl cholinesterase (Ache), Fe receptor type I, and the lymphocyte function-associated antigen 3 (LFA-3). There is a general consensus that the mechanism underlying this deficit resides in the glycolipid-anchor pathway, and it is interesting to notice that our data on LAP mRNA are in agreement with the observation of normal mRNA levels demonstrated for DAF. While DAF is pathophysiologically correlated to PNH since its absence on the surface of PNH red blood cells leads to an increased susceptibility to complement mediated lysis, the role, if any, of the LAP deficit as well as the other PI tail proteins in the pathogenesis of the disease requires further investigation.

**REFERENCES**


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*Results are expressed as MEAN ± SD. The number of individuals analyzed is indicated in parenthesis.
†Results are expressed as MEAN ± SD of two separate determinations.

Fig 1. RNA blotting analysis of LAP transcript in normal, PNH, and stable phase CML PMN. Lanes a, d, e, h: Normal PMN RNA. Lanes b, c: PNH PMN RNA from patients 1 and 2 respectively. Lanes f, g: Stable phase CML PMN RNA from patients 1 and 2 respectively. Lanes a through d; e, f; and g, h represent three separate experiments.
globinuria are deficient in the complement regulatory protein, decay accelerating factor. Proc Natl Acad Sci USA 80:5066, 1983


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