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.

RESULTS

LAP enzyme activity in PNH and CML. Table I 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 I). Thus, LAP scores and enzymatic activity are in agreement and demon-

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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 and values of I .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.

90% of their PMNs.

all have highly depressed levels of LAP activity in more than different steady-state levels of LAP mRNA. PNH PMN this pathological situation since equivalent levels transcript are observed in both control and CML RNA (lanes e, 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).

**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 III, 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 pathophysioologically 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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**Table 1. LAP Score and LAP Activity in CML and PNH**

<table>
<thead>
<tr>
<th>Patient</th>
<th>LAP Score</th>
<th>LAP Activity (units/50 x 10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>20-105</td>
<td>370 ± 133* (4)</td>
</tr>
<tr>
<td>CML</td>
<td>Patient 1</td>
<td>9</td>
</tr>
<tr>
<td>Patient 2</td>
<td>16</td>
<td>13 ± 1†</td>
</tr>
<tr>
<td>PNH</td>
<td>Patient 1</td>
<td>12</td>
</tr>
<tr>
<td>Patient 2</td>
<td>14</td>
<td>13 ± 2†</td>
</tr>
</tbody>
</table>

*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.

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![RNA blotting analysis of LAP transcript in normal, PNH, and stable phase CML PMN](image-url)
globinuria are deficient in the complement regulatory protein, decay accelerating factor. Proc Natl Acad Sci USA 80:5066, 1983
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