CONCISE REPORT

$c\text{-myc}$ and $c\text{-fos}$ Expression During Interferon-$\alpha$ Therapy for Hairy Cell Leukemia

By Pierre Lehn, François Sigaux, David Grausz, Pascale Loiseau, Sylvie Castaigne, Laurent Degos, Georges Flandrin, and François Dautry

Low-dose interferon-$\alpha$ (IFN-$\alpha$) therapy is consistently effective in the treatment of hairy cell leukemia (HCL). In two cases of resistance to IFN-$\alpha$ administration, we diagnosed variant HCL, a form of HCL with intermediate features between typical HCL and B cell prolymphocytic leukemia. We tried to distinguish variant and typical hairy cells (HCs) by Northern blot analysis of the oncogenes expressed in vivo. We report that variant HCs contain $c\text{-myc}$ transcripts in contrast to typical HCs, whereas $c\text{-fos}$ transcripts are detected in both cell types. We also report that the mRNA levels of $c\text{-myc}$ are not modified in variant HCs by IFN-$\alpha$ treatment, whereas the level of $c\text{-fos}$ mRNA is modulated in both types of HCs. Our findings suggest that the failure to modulate $c\text{-myc}$ expression in vivo might indicate the limits of low-dose IFN-$\alpha$ therapy.

© 1986 by Grune & Stratton, Inc.

MATERIALS AND METHODS

Hairy cell samples. After informed consent, human samples were obtained. We indicate in Table 1 the hematologic data of the patients before IFN-$\alpha$ therapy as well as the origin and the composition of the cell samples obtained by Ficoll-Hypaque density sedimentation. We restricted our study to the rare HCL patients with hyperleukocytosis (the two variant HCL cases and five out of more than 50 typical HCL patients included in our clinical trial evaluating the efficacy of IFN-$\alpha$ therapy) to ensure high levels of purity of the cell samples from the peripheral blood and to provide enough HCs for the Northern blot investigation. We also analyzed two splenic HCs samples obtained from patients undergoing splenectomy and not subjected to IFN-$\alpha$ therapy. Finally, we studied peripheral blood samples obtained during low-dose IFN-$\alpha$ therapy. Because we analyzed four typical HCL cases immediately after the first administration of IFN-$\alpha$ and because IFN-$\alpha$ therapy was ineffective in the two cases of variant HCL, neither the hematologic data nor the composition of the cell samples was significantly modified at the time of RNA extraction. The diagnosis of either typical or variant HCL was assessed by morphologic criteria. In all cases, HCs reacted with antibodies against HLA-DR, B cell–restricted antibodies (B1 and B4), and with the anti-HC monoclonal antibody Leu-M5 (oSHCL3).

Northern blot analysis. Total cellular RNA was extracted by the guanidinium-cesium chloride method and analyzed by electrophoresis on 1.2% agarose-formaldehyde gels followed by Northern blot transfer to nitrocellulose and hybridization to the one probes radiolabeled by nick translation. Hybridization was performed at 68°C in 4x SSPE (1x SSPE: 0.18 mol NaCl, 10 mmol sodium phosphate, pH 7.0, and 1 mmol Na2 EDTA) containing 0.2% sodium dodecyl sulfate (SDS), 0.1% sodium pyrophosphate, and 5 µg/mL of heparin. The filters were washed for one hour each in 1x SSPE + 0.1% SDS and 0.5x SSPE + 0.1% SDS at 68°C before autoradiography. The v-fos probe was the BglII-PvuII fragment of v-fos, and the c-myc probe was the ClaI-EcoRI fragment of the human c-myc gene containing the third exon.

Several precautions were taken to ensure that RNA samples from the different patients could be compared. Ethidium bromide was used to stain each gel to confirm that equal amounts of RNA were loaded on the gel and to control the transfer of RNA from the gel to the nitrocellulose filter. The same control RNAs (HL60, K562, Ramos, and Mol4) were included on all blots and used as internal controls for determining the level of protooncogene expression by the

From the Division of Hematology, Hôpital Saint-Louis, Paris; and the Laboratory of Molecular Oncology, Institute Gustave Roussy, Villejuif, France.

Supported by a grant (ATP Bases fondamentales de la Cancérologie) from Centre National de la Recherche Scientifique and by Grant 85-0497 from Fondation contre la Leucémie.

Submitted Feb. 20, 1986; accepted June 20, 1986.

Address reprint requests to Dr Pierre Lehn, Laboratoire Central d’Hematologie, Hôpital Saint-Louis, 2 place du Dr Fournier, 75475 Paris CEDEX 10, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1986 by Grune & Stratton, Inc.

0006-4971/86/6804-0023$03.00/0


967
Table 1. Initial Hematologic Data and Composition of Cell Samples

<table>
<thead>
<tr>
<th>Patients</th>
<th>Diagnosis</th>
<th>Splenectomy</th>
<th>Origin of Cells</th>
<th>Initial Blood Counts WBC (10^9/dL)</th>
<th>Cell Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood</td>
<td>WBC (10^9/dL)</td>
<td>HCs (%)</td>
</tr>
<tr>
<td>1</td>
<td>Typical HCL</td>
<td>–</td>
<td>Blood</td>
<td>18.3</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>Typical HCL</td>
<td>–</td>
<td>Blood</td>
<td>17.3</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>Typical HCL</td>
<td>+</td>
<td>Blood</td>
<td>59.5</td>
<td>85</td>
</tr>
<tr>
<td>4*</td>
<td>Typical HCL</td>
<td>+</td>
<td>Blood</td>
<td>12.5</td>
<td>54</td>
</tr>
<tr>
<td>5</td>
<td>Typical HCL</td>
<td>+</td>
<td>Blood</td>
<td>13.1</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>Typical HCL</td>
<td>+</td>
<td>Spleen</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Typical HCL</td>
<td>+</td>
<td>Spleen</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Variant HCL</td>
<td>+</td>
<td>Blood</td>
<td>43.0</td>
<td>78</td>
</tr>
<tr>
<td>9</td>
<td>Variant HCL</td>
<td>–</td>
<td>Blood</td>
<td>217.0</td>
<td>97</td>
</tr>
</tbody>
</table>

*IFN-α therapy: 1. 3 x 10^8 units daily of recombinant IFN-α2A from Hoffmann-La Roche Inc, Basel, Switzerland; 2. 2 x 10^8 U/m2 three times each week of recombinant INF-α2 from Schering Corp. Kenilworth N.J.

†In case 4. the mononuclear cells were depleted of T lymphocytes by sheep erythrocyte rosetting.

Results and Discussion

We could detect c-myc transcripts only in the variant HCs. These transcripts are of the normal size. Only in sample 9 is their level as high as that seen in the control cell lines K562, Ramos, and Molt4, but it remains lower than in the H60 cell line (Fig 1B). Contrary to c-myc, normal-sized (2.2 kb) c-fos transcripts are present in HCs from all patients tested, although at variable levels (Fig 1A). The level of c-fos mRNA is typical HCs seems to depend on the presence or absence of the spleen (Fig 1A and Table 1). In addition (data not shown), we could not detect c-myb transcripts in both types of HCs, although they were present in two of the control cell lines, HL60 and Molt4. Harvey-ras mRNA accumulates at similar levels in the HCs and in the control cell lines; this is consistent with previous studies of Ha-ras gene expression in acute and chronic leukemias. Finally, we could not detect c-sis messengers. This is of particular interest because of the relationship between c-sis protein and platelet-derived growth factor (PDGF) and the well-known myelofibrosis in HCL.

Our detection of c-myc transcript exclusively in variant HCs suggests that variant HCL is a more proliferative disease than typical HCL. Because we could also detect
c-myc transcripts in two cases of B cell prolymphocytic leukemias (data not shown), our results strengthen the classification of variant HCL as an intermediate disorder between typical HCL and B cell prolymphocytic leukemia.7 The heterogeneity of these rare intermediate diseases, already reflected in the difference in the c-myc mRNA levels in our two cases, could be further analyzed by the study of additional cases.

Our findings of c-fos expression in both typical and variant HCs could be related to their mature phenotype since a stable expression of c-fos has been reported only in some differentiated cells.12 In the hematopoietic system in which a stable expression of c-fos is known in macrophages,12,13 our results extend the domain of investigation to the B lymphocyte lineage. Recent observations indicate that c-fos transcripts can be detected during activation of normal human peripheral blood lymphocytes.18,19 In addition we observed noticeable c-fos mRNA levels in prolymphocytic leukemias (data not shown).

To assess further the possible link between c-myc expression in variant HCs and their resistance to IFN-α therapy, we studied the c-myc mRNA levels during treatment. We observed that even after 6 weeks of IFN-α administration, c-myc transcripts persisted at constant levels in variant HCs (Fig 2C), although they remained undetectable in typical HCs at several time points following the first administration of IFN-α (data not shown). In Fig 2B, we also show that the level of c-fos mRNA in variant HCs was modified despite the ineffectiveness of IFN-α therapy. In four typical HCL cases in which IFN-α was effective, a sequential study revealed two patterns of c-fos expression after the first administration of IFN-α (Fig 2A). In two nonsplenectomized patients, we noticed a rapid disappearance of the c-fos transcripts, whereas IFN-α induced a transient accumulation of c-fos mRNA in two splenectomized patients. These variations of c-fos mRNA levels are not due to a generalized change in the overall level of mRNA as indicated by the nearly constant levels of β-actin mRNA (Figs 2A and 2D).

Thus, the expression of c-fos, but not c-myc, in variant HCs is modulated by IFN-α administration. The c-myc gene is of particular interest because not only is its expression closely associated with cellular proliferation but it also has been shown in vitro that in the case of growth inhibition of cell lines by IFN, a decrease in c-myc mRNA accumulation correlates with the antiproliferative activity.20 Therefore, the failure to modulate c-myc expression might be a good indicator of the limits of low-dose IFN-α therapy, even if c-myc expression is not solely responsible for the resistant phenotype. The meaning of the variation of c-fos expression in response to IFN-α remains unclear.

---

**Fig 2.** Expression of c-fos and c-myc during IFN-α therapy. c-fos expression (A) is shown in four typical HCL cases (patients 1, 2, 3, and 4 in Table 1) at the indicated hours following the first administration of IFN-α. c-fos (B) and c-myc (C) expression are depicted in the two variant HCL patients before (−) and after (+) 6 weeks of IFN-α therapy. The arrows indicate the position of the ribosomal RNAs. β-Actin mRNA levels are also shown.
In conclusion, the study of c-myc expression will be helpful in the identification of variant HCLs. Our observations suggest that the particular sensitivity of typical HCs to low-dose IFN-α therapy is related to their very low level of proliferation because a specific event of the G0-G1 phase might be the target of IFN action. Thus, although one cannot preclude other mechanisms, we suggest that the cytostatic effect of IFN-α on typical HCs leads to the progressive disappearance of the leukemic cell population by impairing its renewal. Recent data showing that HCs synthesize B cell growth factor (BCGF) and that IFN-α inhibits BCGF-induced proliferation of HCs are consistent with our hypothesis.

ACKNOWLEDGMENT

We thank Dr R. Monier for helpful discussions and careful reading of the manuscript. We also thank Drs I. Verma, D. Strehlin, and M. Buckingham for providing v-fos, human c-myc, and mouse β-actin plasmids, respectively. We are grateful to M. Dewes for expert secretarial assistance.

REFERENCES


From www.bloodjournal.org by guest on August 27, 2017. For personal use only.
c-myc and c-fos expression during interferon-alpha therapy for hairy cell leukemia

P Lehn, F Sigaux, D Grausz, P Loiseau, S Castaigne, L Degos, G Flandrin and F Dautry

Updated information and services can be found at: http://www.bloodjournal.org/content/68/4/967.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml