Interferon-stimulated Genes in Interferon-sensitive and -resistant Chronic Myelogenous Leukemia Patients

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ABSTRACT

α-Interferon induces hematological and cytogenetic remissions in some individuals with newly diagnosed Philadelphia-positive chronic myelogenous leukemia. However, interferon-resistant disease occurs in a consistent patient subset (primary resistance) and develops during therapy in additional patients (secondary resistance). Several α-interferon-inducible genes have been characterized. In interferon-resistant cell line variants, defects in these genes have been implicated in the mechanisms mediating resistance. We have, therefore, evaluated mRNA expression of four interferon-stimulated genes (ISGs) following α-interferon therapy. Twenty-seven chronic myelogenous leukemia patients (ten interferon-sensitive patients, 17 interferon-resistant patients) were studied. Peripheral blood samples were collected prior to and 1 to 7 days after starting interferon therapy and analyzed for the expression of 2'-5' oligoadenylate synthetase; ISG-15, ISG-54, and 6-16 transcripts. Following therapy with α-interferon, 2'-5' oligoadenylate synthetase, ISG-54, and 6-16 transcripts were discerned in all patients regardless of their response to interferon. The ISG-15 message was detected in eight of nine interferon sensitive patients and in 15 of 16 interferon-resistant patients, as well. Overall, no consistent defect in the ISG system could be identified. Therefore, lack of induction of these genes cannot explain resistance to α-interferon in chronic myelogenous leukemia patients. Other mechanisms such as posttranslational modification, leading to defects in the ISG corresponding proteins, may play a role in the development of resistance.

INTRODUCTION

IFNs are a family of naturally occurring cytokines produced by eukaryotic cells in response to stimuli such as viruses, mitogens, and a variety of antigens. They are grouped into type I (α and β) and type II (γ) interferons based on physiochemical and antigenic characteristics (1).

The prodigious array of biological activities of α-interferon includes antiviral and cell growth-inhibitory effects. These diverse properties are mediated through binding to a high-affinity cell surface receptor (2). This leads to a variety of intracellular signals which can initiate the rapid execution of functional changes in expression of genes commonly known as ISGs (3-5). These ISGs include genes with well-defined functions [2',5' A synthetase (6, 7), methalothionein II (8), Class I histocompatibility antigens, and interferon-specific protein kinase P-68 (9)], as well as genes 1-8, 6-16, 6-26, and 9-27 and ISG upstream to the transcription initiation site. This 5' flanking sequence, designated the ISRE, is a positively acting interferon enhancer element and is a binding site for putative activator and repressor proteins (3, 6, 12, 13).

Little is known about mechanisms generating the antiproliferative effect of IFN-α. Also, it is not known whether divergent intracellular pathways lead to antiproliferative versus antiviral effects. Recently established interferon-resistant sublines derived from interferon-sensitive [Daudi (lymphoma), Friend (erythroleukemia), and mouse 3 (lymphoma)] cell lines (14-19) have been probed for intracellular changes associated with the development of cellular resistance. Although experimental results have varied, most investigators failed to identify a defect in receptor-mediated binding, and all identified some, though not always the same, defects in the ISG system. Complete failure to induce ISGs following exposure to α-interferon was noted in a cell variant cloned from an interferon-sensitive mouse B-cell lymphoma cell line resistant to both the antiproliferative and antiviral effects of α-interferon (18). Only partial failure to induce ISGs was noted in most of the other studies (8, 14, 19). These data indicate that, in cell lines, defects in ISG transcription are associated with the development of resistance to the antiproliferative effect of interferon.

Clinical resistance to a cytokine such as interferon may be mediated through a cellular defect similar to the one conferring resistance in a cell line. Alternatively, α-interferon may possess effects on the cellular immune system, i.e., natural killer cell activation, which may play a role in mediating a clinical response, and defects in such a system could also contribute to clinical resistance (18).

Because of the heterogenous responses to α-interferon (20, 21), chronic myelogenous leukemia provides an excellent model to study the mechanisms mediating clinical resistance to interferon. We have, therefore, examined ISG induction in both IFN-α-resistant and -responsive CML patients.

MATERIALS AND METHODS

Peripheral blood was collected by antecubital venous puncture from 27 patients with Philadelphia-positive CML. Samples were collected before therapy, and at one or more time points (24 and/or 48 h, ± 7 days) after initiating α-interferon injections. These time points were chosen because there is no significant change in the WBC elements within 48 h (and often within 7 days) after starting α-interferon, even in responding patients. Treatment was given daily by the s.c. or i.m. route. Doses are shown in Table 1. All patients gave informed consent according to institutional guidelines. The disease characteristics of these patients, treatment doses of IFN-α, and the type of response are outlined in Table 1. The patients were treated with either rIFN-α-2a (Roferon-A; Hoffmann-LaRoche, Nutley, NJ) or rIFN-α-2b (Interon; Schering-Plough, Kenilworth, NJ).

Eighteen of the patients were studied at the time of therapy onset (Table 1), and their clinical response was assessed subsequent to the study. The remaining nine patients were studied during the course of therapy when their response to interferon was already defined and...
INTERFERON GENE STIMULATION IN CML

Table 1 Clinical characteristics of α-interferon-treated chronic myelogenous leukemia patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Response</th>
<th>IFN starting dose (x 10^5 units/day)</th>
<th>Time from treatment onset to study (mos.)</th>
<th>WBC pretherapy (x 10^5/µl)</th>
<th>WBC posttherapy (x 10^5/µl)</th>
<th>Date of therapy</th>
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* Secondary resistance (a); primary resistance (b).
*—, study of gene induction coincides with therapy onset.
*—,—, patient continuing therapy with IFN-α.
* Patient continuing therapy with hydroxyurea + IFN-α.

following treatment interruption for periods ranging from 3 days to 3 mos.

Response Criteria. Patients were defined as interferon responsive if they achieved complete or partial hematological remission (22). Patients who failed to demonstrate a decrease in their WBC count to a level consistent with partial hematological response and those who demonstrated an increase in count during the course of therapy were defined as having interferon-resistant CML. They were further characterized as having either primary resistance, if their disease failed to respond to interferon from the outset, or secondary resistance, if they achieved complete or partial hematological remission (22). Patients who failed to demonstrate a decrease in their WBC count to a level consistent with partial hematological response and those who demonstrated an increase in count during the course of therapy were defined as having interferon-resistant CML. They were further characterized as having either primary resistance, if their disease failed to respond to interferon from the outset, or secondary resistance, if they demonstrated an initial response which was subsequently lost (Table 1).

Preparation of RNA and Blot Hybridization. Assays were carried out onuffy coat cells obtained from patients with CML. Cells were lysed with 4 M guanidinium isothiocyanate (Bethesda Research Laboratories, G. Stark, Imperial Cancer Research Fund Laboratories; 2',5' oligo (A) separated in 1% agarose/formaldehyde gels, transferred to nitrocellulose filters, and verified, by autoradiography, disappearance of the signal of the former probe. Preparation of cDNA probes and hybridization conditions used for the Northern analysis have been previously described (25).

DNA Probes. cDNA probes included ISG-15 (11) and ISG-54 (3) donated by Dr. D. Levy of New York University; 6-16(12) donated by Dr. J. Chebath of The Weizmann Institute of Science; and β-actin (American Type Culture Collection, Rockville, MD). T-Cell receptor Cβ probe was donated by J. Kagan, M. D. Anderson Cancer Center.

RESULTS

The expression of four interferon-stimulated genes prior to and during therapy was studied in 27 patients. Ten patients were sensitive to α-interferon, and 17 patients were resistant. The characteristics of these patients are outlined in Table 1.

Expression of 2',5'A Synthetase. At least four different mRNA messages are known to exist for 2',5'A synthetase (1.6-, 1.8-, 2.4-, and 3.6-kilobase mRNA, respectively) (26, 27). In 3 patients, 2',5'A synthetase mRNA was expressed prior to the initiation of α-interferon therapy, albeit at very low levels. Following therapy, 2',5'A synthetase mRNA was induced in all 27 patients. The 1.6-kilobase mRNA was expressed in 10 patients (7 interferon resistant and 3 interferon sensitive). The 1.8-kilobase mRNA was expressed in 14 patients (10 with resistant disease and 4 with interferon-sensitive disease). Only in three cases were both lower weight messages coexpressed (Fig. 1). The 2.4-kilobase mRNA was expressed in 14 patients, 10 with interferon-resistant disease and 4 with interferon-sensitive CML. And finally, the 3.6-kilobase 2',5'A synthetase mRNA was induced in 6 patients, 4 of whom had interferon-resistant disease.

Low-level messages for ISG-54 were detected prior to therapy in 8 of the patients (5 with interferon-resistant disease and 3 with interferon-sensitive disease). However, following therapy we noted either de novo induction or increased expression of ISG-54 in all 26 patients studied (10 with interferon-sensitive disease and 16 with interferon-resistant disease) (Fig. 1).

The message for ISG-15 was detected prior to therapy in 4 patients (3 with interferon-resistant disease and 1 with interferon-sensitive disease) (Fig. 2). However, de novo induction or augmented expression of the mRNA for the ISG-15 was detected in 23 of 25 studied patients (15 with interferon-resistant disease and 8 with interferon-responsive disease). One responding patient and one interferon-resistant patient failed to demonstrate induction of ISG-15.

Low levels of 6-16 gene mRNA were detected in 7 patients (5 with interferon-resistant disease and 2 with interferon-responsive disease) prior to therapy. Following therapy, the 6-16...
mRNA expression was either induced de novo or increased in expression in all 27 patients studied, including 17 with interferon-resistant disease and 10 with disease that responded to therapy (Fig. 2).

All blots were rehybridized with a β-actin probe to verify equal RNA loading of gels (Figs. 1 and 2). In addition, after washing, blots were hybridized with a T-cell receptor Cγ probe. While phytohemagglutinin-stimulated T-cells were positive for signal, no signal was seen in any patient sample (data not shown).

**DISCUSSION**

In this study of CML Ph1 patients with interferon-responsive and interferon-resistant disease, we demonstrated no difference in mRNA induction of interferon-stimulated genes 2',5'A synthetase, ISG-15, ISG-54, and 6-16 between interferon-sensitive and -resistant patients. The possibility of contamination by nonleukemic cells is virtually ruled out by lack of T-cell receptor Cγ expression, which indicates that only a small minority of the cells are T-cells, which may arise from a normal hemopoietic stem cell. In previous work, we failed to demonstrate altered interferon receptors in the interferon-resistant patient subset (28). Subsequently, we showed that 2',5'A synthetase enzymatic activity is induced in interferon-sensitive but not in interferon-resistant CML patients following interferon therapy (29). This finding does not match our current findings, a discrepancy which may result from either posttranslational modification of the 2',5'A synthetase pathway in the resistant cells or alteration in the 2',5'A synthetase isoenzyme profile to a 2',5'A synthetase isoenzyme form with altered requirements for doubled-stranded RNA in the enzymatic assay (27). Further study of 2',5'A synthetase protein steady-state levels and enzymatic activity will be required to settle this issue.

Our failure to detect defects in ISG induction in interferon-resistant patients differs from observations characterizing such defects in various interferon-resistant cell lines (8, 14, 18, 19). It should be stated, however, that although the ISGs studied are commonly involved with cellular defects associated with resistance, many additional ISGs have been identified and may eventually demonstrate defects associated with clinical resistance.

Clinical resistance to interferon in CML Ph1 is defined as a lack of cytoelective effect of α-interferon on the peripheral WBCs or an actual increase in the WBCs despite continuous therapy with α-interferon. It is not clear whether this is an absolute or relative resistance and whether dose escalation beyond standard treatment doses will circumvent this type of resistance. Considering the fact that patients are already treated at maximally tolerated chronic doses of interferon (20, 21, 30), this question cannot be answered easily by further therapy dose escalation.

Interferon possesses multiple biological activities, and more than one of these may play a role in CML. A direct antiproliferative effect on the malignant myeloid cell is the most plausible mode of action in CML. However, mechanisms such as activation of natural killer cells or the correction of CML cell defects in adhesion to stroma may be important in the clinical response to interferons. Corresponding defects in cellular responses to interferon may not be related to defects in the ISGs studied in this work (31–34).

Finally, recent studies have demonstrated interferon-mediated antiproliferative effects following heterologous modulation of growth factor receptors and alteration of growth factor-induced mitogenic response (35–38). The possibility that α-interferon modulates myeloid stem cell response to hemopoietic growth factors has not been studied. It is believed, however, that CML Ph1 is a disease which undergoes multiple cellular alterations. It is possible that some of these alterations involve transition from growth factor dependence to autonomous growth because of autocrine production of growth factors or inactivation of suppressor genes (39). We have recently demonstrated the autocrine and paracrine generation of interleukin 1β in the advanced stages of CML Ph1 but not in the early chronic phase (40). Accordingly, it is conceivable that some α-interferon actions, such as ISG induction, are not defective in the resistant disease; instead, the resistant cell has overridden the inhibitory effect of interferon by the aberrant production of growth factors or by altered response to exogenous growth factors.
REFERENCES


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