CONCISE REPORT

Molecular Analysis of Interferon-Induced Suppression of Philadelphia Chromosomes in Patients With Chronic Myeloid Leukemia

By G. Yoffe, M. Blick, H. Kantarjian, G. Spitzer, J. Gutterman, and M. Talpaz

Treatment with recombinant human interferon alpha-A (Roferon-A) is associated with stable suppression of the population of cells that display the Philadelphia (Ph') chromosome in some patients with chronic myelogenous leukemia (CML) as defined by cytogenetic analysis. Southern blot analyses employing a 3' breakpoint cluster region (bcr) probe (Pr-1) were performed to confirm a complete suppression of the Ph' chromosome-positive clone of cells at the DNA level. The complete disappearance of rearranged restriction fragments of the bcr gene, which were characteristic of the disease prior to Roferon-A therapy, was accompanied by the restoration of normal bone marrow and achievement of durable ongoing complete remission for 9 and 6 months, respectively, in two patients with Philadelphia-positive (Ph') CML. Molecular analysis is a valuable probe for monitoring the clinical course of disease in patients with Ph' CML.

THE ANTIPROLIFERATIVE activity of human alpha interferon has been demonstrated in a number of human tumors in vivo as well as in normal myeloid and myeloid leukemic progenitor cells in vitro. Recombinant human interferon alpha-A (Roferon-A) has recently been shown to induce hematologic remission in most of the patients with chronic myelogenous leukemia (CML) in the early benign phase. Therapy with Roferon-A resulted in a durable Philadelphia (Ph') chromosome suppression (cytogenetic) in some of these patients. Other studies have also demonstrated a complete, albeit transient, suppression of the Ph' clone of cells following intensive chemotherapy.

Because more than 90% of the cases of CML display the Ph' chromosome, which is the result of a 9;22 translocation, this constitutes a hallmark of the disease. The c-abl oncogene, located on chromosome 9, and the breakpoint cluster region (bcr) gene, located on chromosome 22, are juxtaposed in the Ph' CML. Thus producing a bcr/abl chimeric mRNA transcript and a chimeric fusion protein. Upon the introduction of molecular probes capable of identifying this alteration, molecular analysis along with cytogenetic analysis can be used for monitoring therapies with interferon, intensive chemotherapy, and other treatment modalities throughout the course of the disease in patients with CML.

In this study, suppression of the Ph' chromosome clone of cells in two patients with Ph' CML following treatment with Roferon-A was accompanied by the loss of an aberrant restriction fragment of the bcr gene, which was characteristic of the disease prior to Roferon-A therapy. This provides evidence at the DNA level for suppression of the Ph' clone of cells in two patients with CML treated with Roferon-A.

MATERIALS AND METHODS

Case Reports

Patient 1. The diagnosis of Ph' CML was made in this 32-year-old white female in June 1984. She was treated initially with hydroxyurea for 2 months, and in October 1984, daily intramuscular injections of 5 x 10^6 U/m^2 of Roferon-A were started. Prior to treatment with Roferon-A, the patient's peripheral WBC was 18.8 x 10^9/L, and her platelet count was 566 x 10^9/L. The bone marrow cellularity was 100%, and 100% of the cells were Ph'. The hematologic parameters of her disease improved gradually, and hematologic remission was achieved 7 weeks after the Roferon-A therapy was started. At that time, her WBC decreased to 3.4 x 10^9/L, and her platelets decreased to 125 x 10^11/L. Subsequently, after 14 weeks of therapy, the treatment dose of Roferon-A was reduced to 2.5 x 10^6 U/m^2 when the bone marrow cellularity decreased to 40% and cytogenetic analysis demonstrated a decrease of the Ph' cells to 70%. The Ph' clone of cells decreased further to 30% at 6 months and to 10% at 9 months of therapy. Complete suppression of the Ph' clone of cells occurred after 12 months of therapy and has been sustained after 15 and 18 months after therapy was started.

DNA Isolation and Southern Blotting

Blood and bone marrow samples were obtained from patients after they had been fully advised of the procedure and attendant risks in

From the Departments of Hematology and Clinical Immunology and Biological Therapy, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute at Houston. Submitted September 24, 1986; accepted November 19, 1986.

Supported in part by the James E. Lyon Foundation and the Clayton Foundation for Research and Grant CA 31356 from the National Cancer Institute, Department of Health, Education, and Welfare (G.S.). J.G. is a Senior Clayton Foundation Investigator.

Dr Yoffe is now affiliated with the Departments of Pediatrics and Hematology/Oncology, Southwest Medical School, University of Texas Health Science Center at Dallas.

Address reprint requests to M. Blick, DO, Clinical Immunology, Box 41, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

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.
accordance with institutional guidelines and had given informed consent. High-molecular weight DNA was extracted from peripheral blood leukocytes and bone marrow cells by methods described elsewhere. The digested DNA was electrophoresed in a 0.8% agarose gel in a buffer containing 0.4 mol/L Tris, 20 mmol/L EDTA, and 100 mmol/L Na acetate, denatured, neutralized, and transferred to nitrocellulose filters by the Southern blot method. Prehybridization and hybridization were carried out as described previously. A bcr probe (Pr-I) purchased from Oncogene Sciences (Mineola, NY) and corresponding to the breakpoint cluster region on chromosome 22 was labeled to a specific activity of 1 to 3 x 10^8 cpm/μg of DNA by nick translation. Following hybridization, the filters were washed under stringent conditions, air-dried, and exposed to x-ray film for one to five days at -70 °C.

RESULTS

High-molecular weight DNA was obtained from the peripheral blood of both patients before treatment with Roferon-A and from the peripheral blood of a normal donor. The DNAs were digested by BamHI and BglII restriction endonucleases and analyzed by Southern blot using a 3' bcr probe (Pr-I). Analysis of the DNA from patient 1 demonstrated abnormal 11-kb BamHI and 9-kb BglII hybridizing fragments in addition to the normal 3.3-kb BamHI and 5-kb BglII bcr fragments (Fig 1, panel 1). Similarly, patient 2 disclosed abnormal 7.6-kb BamHI and 9-kb BglII hybridizing fragments in addition to the normal bcr restriction fragments (Fig 1, panel 2). Repeat analysis of the DNA from both patients' peripheral blood was carried out on samples obtained 12 months after Roferon-A therapy was started. This analysis disclosed that the rearranged hybridizing fragments were not detected by Southern blot using the same bcr probe (Fig 2, panel 2). Twelve months after Roferon-A therapy was started, bone marrow DNA was also obtained from patient 1, and a similar Southern blot analysis disclosed no bcr rearrangement (Fig 2, panel 1). To determine the sensitivity of our Southern blot assay, a mixing experiment was performed (Fig 3). Using DNA from a normal donor and DNA from a CML patient with an additional abnormal 3-kb BglII restriction fragment, we found that as little as 5% contamination of DNA from the Ph+ cells was clearly detectable as a faint band after a long exposure (120 hours). Therefore, in this assay, the level of detection was approximately 5% by Southern blot analysis.

DISCUSSION

We have demonstrated the disappearance of the Ph+ clone of cells in two patients with CML following treatment with Roferon-A. In addition to cytoconversion of the Ph+ chromosome in all analyzed metaphases, a rearranged bcr DNA fragment characteristic of the patients' disease prior to Roferon-A therapy completely disappeared at a time coincident with hematologic remission.

Previous studies have demonstrated the restoration of nonclonal hematopoiesis in patients with CML following intensive chemotherapy. Similarly, we have reported partial and complete suppression of the Ph+ clone after alpha interferon therapy. In this study, we confirm that the clone.
of cells bearing a DNA rearrangement associated with the formation of the Ph¹ chromosome in CML disappeared from the patients' blood and bone marrow. We have further demonstrated that as little as 5% Ph¹ cells in any given sample can be detected by using Southern blot analysis. The possibility exists that the Ph¹ clone was still present in less than 5% of the bone marrow cells.

Although the Ph¹ chromosome and rearranged bcr gene are associated with more than 90% of the cases of CML, it is still unknown whether suppression of the clone bearing the rearranged gene and the Ph¹ chromosome restores the bone marrow to a normal polyclonal pattern. In this instance, a second clonal marker such as the glucose-6-phosphate dehydrogenase isoenzyme pattern or the restriction fragment length polymorphism of the hypoxanthine-guanine phosphoribosyl-transferase gene may help determine whether the
cells that remain when patients are in remission represent the same abnormal clone and simply lack the Ph¹ chromosome and the bcr rearrangement. However, since the clinical and hematologic features of CML disappeared completely in these two patients, it is unlikely that the hematopoietic cells of these patients in remission still consist of a monoclonal population.

Demonstration of the suppression of the Ph¹ clone by cytogenetic analysis may reflect only the events in a specific cell population that happens to enter mitosis. Molecular analysis, however, circumvents this problem and provides information on the total cell population (dividing and nondividing cells as well). Therefore, the demonstration of complete suppression of an existing gene rearrangement supports the notion that these patients with CML were in complete remission at the molecular level.

REFERENCES


Molecular analysis of interferon-induced suppression of Philadelphia chromosome in patients with chronic myeloid leukemia

G Yoffe, M Blick, H Kantarjian, G Spitzer, J Gutterman and M Talpaz