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Selective reduction of c-*myc* mRNA in Daudi cells by human β interferon

(mRNA/dot blots/blot hybridization transfer/oncogene)

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ABSTRACT **Under normal growth conditions, the human lymphoblastoid cell line Daudi expresses high levels of c-*myc* mRNA. These cells are also sensitive to growth inhibition by interferons. We have compared the levels of mRNA for the c-*myc* in untreated and human β interferon (IFN-β)-treated Daudi cells by RNA dot-blot and blot-hybridization analysis methods. Using a synthetic oligonucleotide complementary to the human c-*myc* mRNA as the probe, we detected a more than 75% reduction in the c-*myc* hybridizable poly(A)$^+$ RNA in the IFN-β-treated cells. This reduction in the c-*myc* mRNA appears to be selective because the level of actin mRNA is not significantly affected by the IFN-β treatment. In addition, neither *in vitro* translation of mRNA extracted from IFN-β-treated cells nor *in vivo* synthesis of cellular proteins in IFN-β-treated cells are quantitatively affected. We surmise that the selective reduction in the amount of c-*myc* mRNA in IFN-β-treated Daudi cells may be related to the IFN-induced inhibition of the Daudi tumor cell growth.**

In addition to its antiviral activity, interferon (IFN) has the capacity to inhibit the growth of cells *in vitro* (1) and some tumors *in vivo* (2). Although various mechanisms, primarily involving the inhibition of viral gene expression, have been proposed for IFN's antiviral activity (see ref. 3 for a review), the molecular mechanism responsible for the antiproliferative action of IFN is unknown. Progress toward understanding the antiproliferative activity would be facilitated if the effect of IFN could be determined on the expression of specific cellular genes involved in the regulation of cellular proliferation and/or the process of neoplastic transformation.

We have chosen to study the mechanism of IFN's inhibition of cell growth using human Daudi cells, a line of lymphoblastoid cells derived from an African Burkitt lymphoma (4), whose growth can be inhibited by low concentrations of IFN (5, 6). Daudi cells have a reciprocal translocation between chromosomes 8 and 14 that is characteristic of other Burkitt lymphomas (7). Because of the chromosomal rearrangement, the human cellular homologue (c-*myc*) of the avian myelocytomatosis virus (MC29) oncogene was translocated from its resident chromosome 8 to chromosome 14 and became transcriptionally active (8). An increased expression of the c-*myc* oncogene has been also reported in other Burkitt lymphomas (8, 9), in mouse plasmacytomas (9, 10), and in avian B-cell lymphomas induced by an avian leukosis virus (11). It has been suggested from these studies that the transcriptional activation of the c-*myc* oncogene may be an important step in the pathway to B-cell neoplasia (8–11). As a result of these studies, it was of interest to us to determine whether the expression of the human c-*myc* is affected in Daudi cells, whose growth is inhibited by IFN-β. We report that under such conditions the level of the c-*myc*-specific RNA is reduced more than 75%.

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MATERIALS AND METHODS

Cells and *in Vivo* Labeling. Human Daudi cells were grown in RPMI medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) and gentamicin (GIBCO) at 37°C in a 5% CO_2/95% air incubator. Human IFN-β was produced and purified to homogeneity as described (12). Daudi cell proteins were labeled *in vivo* with [^{35}S]methionine. Cells were collected by centrifugation, washed once with methionine-free medium, resuspended in methionine-free medium containing 100 μCi of [^{35}S]methionine per ml (1100 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) and incubated at 37°C for 3 hr. Cells were broken in a Dounce homogenizer in 10 mM Tris·HCl, pH 7.5/10 mM NaCl/1 mM $MgCl_2$. Nuclei were removed by centrifugation, and an equal volume of cytoplasm was added to an equal volume of gel loading buffer, and the proteins were analyzed by electrophoresis on $NaDodSO_4$ gels by the procedure of Laemmli (13).

RNA Isolation and *in Vitro* Translation. Total RNA was isolated by a modified guanidinium isothiocyanate–cesium chloride method (14). Chloroform/butanol extraction was omitted, and the RNA after the cesium chloride gradient was run immediately on oligo(dT)-cellulose (15). After two cycles of purification, poly(A)$^+$ RNA was precipitated by ethanol and stored under ethanol at −70°C until further use. The concentration of RNA was determined by A_{260} readings, where 1 A unit was assumed to equal 40 μg/ml (14). Preparation of poly(A)$^+$ RNA and the conditions for *in vitro* translation in a rabbit reticulocyte lysate system (Bethesda Research Laboratories) were performed according to the manufacturer's recommendations. Each assay contained 2 μg of poly(A)$^+$ RNA and 50 μCi of [^{35}S]methionine. The translational products were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (13). The gels were exposed to Kodak XRP-5 film with Du Pont Cronex Quanta III intensifying screen at −70°C for 3 days.

Dot-Blot and Blot-Hybridization Analysis. Poly(A)$^+$ RNA was analyzed by dot hybridization as described by Müller *et al.* (16). Briefly, dilutions of poly(A)$^+$ RNA were made in H_2O, heated at 65°C for 10 min, and quick-cooled on ice; 2 μl was spotted on nitrocellulose paper that had been equilibrated with 20× NaCl/Cit (1× NaCl/Cit is 150 mM NaCl/15 mM sodium citrate) and air-dried. After baking for 2 hr at 80°C under vacuum, the blot was prehybridized for 5 hr at 45°C as recommended (16). Hybridization was performed for at least 20 hr at 45°C with 10 ng of ^{32}P-labeled c-*myc* probe (see below) per ml. After hybridization, the blot was washed three times in 1× NaCl/Cit at 50°C for 2 hr.

For blot-hybridization analysis, samples of poly(A)$^+$ RNA were denatured with formaldehyde and fractionated by electrophoresis on 1.4% formaldehyde/agarose gels as described (14) except that the buffer used was 20 mM sodium borate, pH 8.3/1 mM EDTA. After electrophoresis, the gels were rinsed with H_2O; without further treatment, the RNA was

Abbreviation: IFN, interferon.

transferred to nitrocellulose paper with 20× NaCl/Cit (17). The blots were baked in a vacuum oven for at least 2 hr at 80°C and further treated by exposure to 100°C for 5 min in 20 mM Tris·HCl (pH 8.0) (17). The blots were prehybridized and hybridized as described above, but the temperature was lowered to 30°C (for the c-*myc* probe) and to 42°C (for the actin probe). Washing was performed with two changes of 1× NaCl/Cit containing 0.2% $NaDodSO_4$ at 50°C for 15 min.

Preparation of Probes. A synthetic c-*myc* probe of 20 nucleotides in length was prepared by R. Arentzen of the Du Pont Central Research and Development Department. The probe sequence 5′ G-A-C-T-C-T-G-A-C-A-C-T-G-T-C-C-A-A-C-T 3′ is complementary to a segment of the c-*myc* mRNA as predicted from published sequence data (18, 19). This sequence codes for amino acid residues Lys-Leu-Asp-Ser-Val-Arg-Val in the second coding exon of the human c-*myc*. The probe was end-labeled with ^{32}P by using T4 polynucleotide kinase (Bethesda Research Laboratories) and [γ-^{32}P]ATP (3000 Ci/mmol; Amersham) as described (14) to a specific activity of 5–10 × 10^8 cpm/μg.

A plasmid containing cytoplasmic chicken actin cDNA that hybridizes with human actin mRNA was provided by J. E. Darnell of The Rockefeller University. The plasmid was grown, purified, and nick-translated (14) in the presence of [α-^{32}P]dATP to a specific activity of 10^8 cpm/μg.

Quantitation of Autoradiograms. Films from dot-blot and blot-hybridization analysis after different exposures were scanned on a MK III Microdensitometer (Joyce–Loebl). Individual peaks from each tracing were cut out and weighed, and the ratios between corresponding peaks were determined. The individual dots also were cut out from dot blots and assayed for radioactivity in Universal LSC cocktail (Fisher) with a Packard liquid scintillation spectrometer, and the ratios between cpm from corresponding dots were determined.

RESULTS

The effect of IFN-β on our line of Daudi cells is shown in Fig. 1. An effect on cell growth can already be seen 24 hr after the addition of the IFN-β. Growth of the Daudi cells is completely inhibited between 24 and 72 hr of IFN-β treatment.

In order to determine the effect of IFN-β on the level of c-*myc* RNA, we prepared total poly(A)$^+$ RNA from untreated and IFN-β-treated (50 units/ml) Daudi cells after a 24-hr exposure. The RNA was analyzed for sequences hybridizable to ^{32}P-labeled c-*myc* synthetic probe by dot-blot analysis as described. Ten micrograms of the RNA and four successive 1:3 dilutions were spotted on the blot. An autoradiogram of the blot after 7 days of exposure is shown in Fig. 2. The amount of c-*myc*-specific RNA was reduced by a factor of at least a 1:3 dilution in IFN-β-treated cells. Densitometer tracings of the dots revealed that c-*myc* RNA in the IFN-β-treated cells was 1/4th to 1/5th that in the untreated cells. Individual dots also were cut out from the blot and assayed for radioactivity in a scintillation counter. The ratio between cpm from the untreated and IFN-β-treated RNA dots also was 4–5:1. These results were confirmed in two additional independent experiments (data not shown).

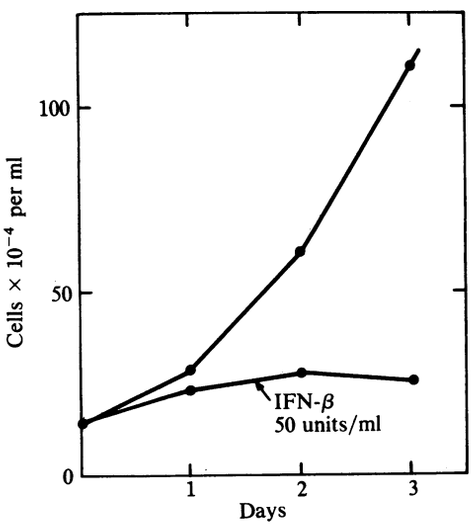

FIG. 1. Effect of IFN-β at 50 units/ml on the growth of Daudi cells. Cells were counted each day.

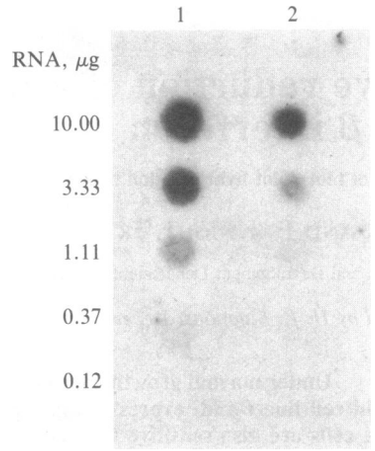

FIG. 2. Dot-blot analysis of c-*myc* expression. Indicated quantities of poly(A)$^+$ RNA from untreated (row 1) and IFN-β-treated (row 2) Daudi cells were spotted on nitrocellulose paper and the blot was hybridized with ^{32}P-labeled c-*myc* synthetic probe at 45°C for 20 hr, washed, and exposed for 7 days.

The same RNA was further analyzed by blot hybridization after separation on a 1.4% agarose-formaldehyde gel. An autoradiogram of this analysis is shown in Fig. 3. The blot was first hybridized to the c-*myc* probe (Fig. 3*A*). Hybridization was detected only to a single broad band in both RNA preparations. The molecular size of the band (2.4 kilobases) is in close agreement with the size of the c-*myc* mRNA in Daudi cells as previously reported (8). Densitometer tracings of the bands of the autoradiograms after different time exposures indicated that the c-*myc* RNA in the IFN-β-treated cells was 1/4th to 1/5th that in untreated cells.

Because IFNs inhibit the growth of Daudi cells, it might be anticipated that a decreased synthesis or an increased degradation of RNA would occur in these cells. We compared *in vivo* labeling of proteins in IFN-treated Daudi cells to that in untreated Daudi cells and compared *in vitro* translation of the mRNAs extracted from IFN-treated and untreated cells. The results (Fig. 4) indicated that, in either the *in vivo* (Fig. 4*A*) or *in vitro* (Fig. 4*B*) systems, there was no significant reduction of active mRNA in cells treated with IFN-β.

Further support of the idea that IFN does not cause a generalized reduction of cellular RNA comes from the experiment depicted in Fig. 3*B*. The same blot used in Fig. 3*A* was stripped of the c-*myc* probe and hybridized with a chicken actin cDNA probe. As determined by densitometric tracing, the amount of actin hybridizable RNA was reduced only slightly (7%) in IFN-β-treated cells.

DISCUSSION

The objective of this work was to determine the expression of the c-*myc* oncogene in Daudi cells whose growth was in-

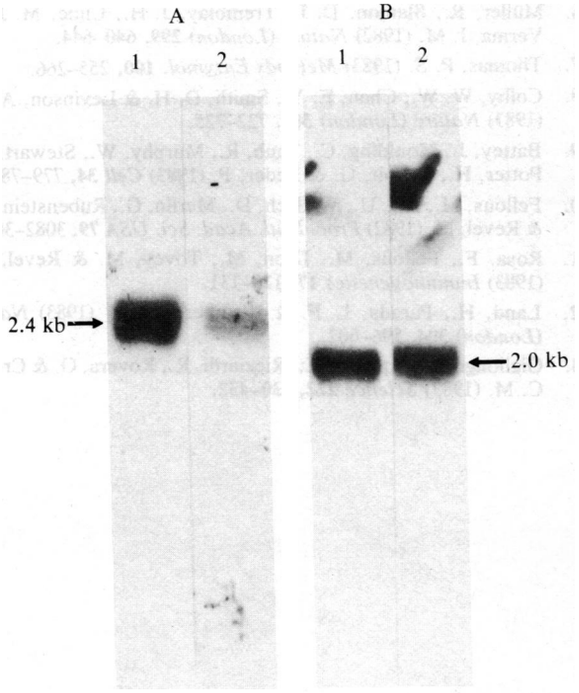

FIG. 3. Blot-hybridization analysis of RNA from Daudi cells. Poly(A)$^+$ RNA (10 μg) from untreated (lane 1) and IFN-β-treated (lane 2) Daudi cells was separated on 1.4% agarose gel containing formaldehyde. The RNA was transferred without further treatment to nitrocellulose paper, and the blot was first hybridized with the ^{32}P-labeled c-*myc* synthetic probe at 30°C for 20 hr, then washed, and exposed for 7 days (*A*). After the exposure, the c-*myc* probe was stripped by boiling the blot for 5 min in H_2O (17) and rehybridized with ^{32}P-labeled chicken actin cDNA probe at 42°C for 40 hr. The blot was washed and exposed for 2.5 days (*B*). kb, Kilobases.

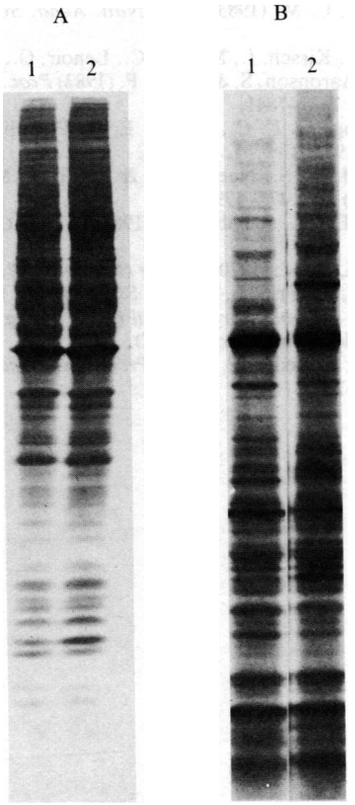

FIG. 4. Effect of IFN-β on the *in vivo* (*A*) and *in vitro* (*B*) synthesis of Daudi cell proteins. Autoradiograms of the [^{35}P]methionine-labeled proteins separated on 10–16% (*A*) and 9–18% (*B*) linear gradient $NaDodSO_4$/polyacrylamide gels. (*A*) Cytoplasmic proteins from untreated cells (lane 1) and from IFN-β-treated cells (lane 2). (*B*) Translational products of mRNA from untreated cells (lane 1) and IFN-β-treated cells (lane 2).

hibited by IFN-β. By both dot-blot and blot-hybridization analyses, we found that the amount of c-*myc*-specific RNA was reduced by more than 75%. The reduction was shown to be selective because *in vivo* labeling of cellular proteins, *in vitro* translation of total cellular mRNA, and the level of actin mRNA were not significantly altered by IFN-β treatment. Our findings are in contrast but not in disagreement with the results of others who studied the effect of IFN on gene expression in Daudi cells. It has been reported that IFN enhances the expression of mRNA for the heavy chain of the HLA antigen (20) and the mRNA of an abnormal β_2-microglobulin (21). We also observed an increased expression of new mRNAs in IFN-β-treated Daudi cells (Fig. 4*B*). The significance of these changes is unknown, but they indicate that the mode of IFN action is complex and may involve both up and down regulation of specific cellular genes.

Whether the reduction in the level of c-*myc* expression is the cause or consequence of the IFN-mediated inhibition of Daudi cell growth cannot be determined from these experiments and is at present a matter of conjecture. It has been suggested that activation of the c-*myc* oncogene may be necessary for the establishment of the neoplastic state of lymphoid cells (8–11). In addition, Land *et al.* (22) have shown that rat embryo fibroblasts became tumorigenic only when cotransfected with both the *ras* and *myc* oncogenes. More recently, Giallongo *et al.* (23) have demonstrated the expression of c-*myc* oncogene in proliferating normal cells. A protein of 48,000 daltons was precipitated with c-*myc*-specific antiserum from lysates of lipopolysaccharide-stimulated mouse spleen cells and from human peripheral blood lymphocytes stimulated with pokeweed mitogen. These observations support the idea that the expression of the c-*myc* oncogene may play an important role in cell proliferation and neoplastic transformation. It would be consistent with this idea to assume that a reduced expression of the c-*myc* may be a factor in IFN's inhibition of cell growth. However, further experiments are clearly necessary to define how essential is the relationship between the steady-state level of c-*myc* mRNA and/or c-*myc* protein and cell growth.

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