Interferon-α Treatment of Daudi Cells Down-regulates the Octamer Binding Transcription/DNA Replication Factors Oct-1 and Oct-2

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The interferons are a group of proteins defined by their ability to inhibit virus infection (1). These factors have also been shown to induce (2) or repress (3) the expression of various genes and also to inhibit the growth of certain cell types (4). A transformed human B lymphoblastoid cell line, Daudi (5, 6), responds to α-IFN† in a number of ways. When these cells are cultured with α-IFN several genes are rapidly induced (7) while the expression of others, including immunoglobulin genes (8), c-fgr (9) and c-myc (7), are inhibited. In addition, the incorporation of exogenous thymidine into DNA declines dramatically and the cell cycle is arrested in Go/G1 at the expense of the S and G2/M phases (10).

The octamer-binding transcription factors Oct-1 and Oct-2 are likely to play a critical role in gene expression and DNA replication in B-cells (for review, see Ref. 11). For instance, the ubiquitous transcription factor Oct-1 is required both for the constitutive transcription of the snRNA genes and the cell cycle-specific expression of the histone H2B gene (12), while the B-cell specific Oct-2 protein also plays an essential role in the expression of the immunoglobulin genes (13, 14).

Moreover, Oct-1 has been shown to be identical to nuclear fraction III, a protein which is essential for the initiation of DNA replication (15–17). It has been shown that both Oct-1 and Oct-2 are capable of stimulating DNA replication in vitro (18), indicating that they are likely to be DNA replication factors as well as transcription factors.

The factors Oct-1 and/or Oct-2 could be involved in mediating the effects of α-IFN on gene expression or on DNA replication in Daudi cells. We therefore investigated the effects of α-IFN treatment on the levels of Oct-1 and Oct-2 activity of octamer-containing gene promoters in the presence and absence of α-IFN.

EXPERIMENTAL PROCEDURES

Cell Culture

Wild-type Daudi cells (Daudi) and the α-IFN-resistant mutant of the same cell line (DaudiR10) were cultured at 3–7 × 10⁶ cells/ml in RPMI-1640 (Gibco-BRL) supplemented with 5% heat-inactivated fetal calf serum. HeLa cells were cultured as a monolayer in Eagle medium, 5% fetal calf serum. Where indicated, α-IFN (Wellferon) was added to a final concentration of 300 units/ml from a stock at 3.0 × 10⁶ units/ml. Phorbol 12-myristate 13-acetate (PMA, Sigma) was added from a 1 mM stock in dimethyl sulfoxide to a final concentration of 100 nM. Addition of dimethylsulfoxide to control extracts had no effect on the cell cycle.

Mobility Shift Assays

Nuclear extracts from 1–5 × 10⁷ cells were prepared from each sample according to the method of Dignam et al. (19). Extracts were incubated with labeled oligonucleotide a (see below) which has a high affinity binding site for Oct-1 and Oct-2 and run on nondenaturing acrylamide gels as previously described (20). Specificity of the Oct-1 and Oct-2 signals was determined by competing the labeled DNA with a 200-fold molar excess of unlabeled oligonucleotide a, with a mutant octamer (ATAATAAT) which does not bind octamer proteins (21) (data not shown), or with an Sp1-binding site (22).

Cell Cycle Analysis and BrdUrd Incorporation

To determine the proportion of cells undergoing DNA synthesis, Daudi cells were cultured with 3 μM bromodeoxyuridine (BrdUrd, Sigma) in RPMI for 0–24, 24–48, and 48–72 h after addition of α-IFN. Control Daudi cells without α-IFN were cultured with BrdUrd for 24 h. Cells were fixed in 70% ethanol for 10 min, resuspended in 1 M HCl for 10 min, then immunolabeled using anti-BrdUrd monoclonal antibody (Becton-Dickinson) and fluorescein isothiocyanate-IgG conjugate (Sigma). Cells were finally stained with propidium iodide (23) and analyzed by flow cytometry (EPICS-C, Coulter) and the proportions of cells which had incorporated BrdUrd during the 24-h labeling period were determined as were the proportions of cells in the G2/M phase of the cell cycle.

Plasmid DNAs

Constructs a–d contained different octamer oligonucleotides cloned upstream of the herpes simplex virus (HSV) thymidine kinase promoter driving the chloramphenicol acetyltransferase (CAT) gene in the vector pBlZXCAT (24). Construct d contains a consensus octamer motif, ATGCAATTA, which is found in immunoglobulin gene promoters and enhancers (21, 25). Construct b contains the octamer-related TAATGARGAT motif, GCGGTAATGAGAT, which is found in the HSV immediate-early 4/5 gene promoter (26). Constructs a and c contain overlapping octamer/TAATGARGAT motifs of the type found in the HSV immediate-early 1 gene (57). Construct e has the
sequence ATGCTAATGAGAT, while that in c is identical except for the substitution of T for G at the 11th position. The Rous sarcoma virus-CAT construct which does not contain an octamer sequence in its promoter is as described by Gorman et al. (28).

**Transfection into Daudi Cells**

10 μg of each DNA construct was transfected into 8 × 10⁶ Daudi cells by electroporation. Exponentially growing cells were pelleted and the conditioned medium was retained. The cells for each transfection were resuspended in 0.25 ml of RPMI 1640, HEPES, 5% fetal calf serum and pipetted into a 0.4-cm electroporation cuvette (Bio-Rad Gene Pulser). The DNA was added to the cells which were then electroporated at 0.25 V, 960 μF (τ = 33–38). These cells were then put into a flask containing 8 ml of the retained medium with a 1-ml wide-necked pipette and then split into 4-ml aliquots in each of two flasks. Cells were left for 4–6 h and then α-IFN (306 units/ml) was added to one of the flasks. The cells were harvested after a further 48 h in culture and cell extracts were equalized according to protein content (29) and then assayed for CAT activity as described by Gorman (30). The activity was quantified by collecting the radioactive areas from the thin layer chromatography plates which were then subjected to scintillation counting. The data is presented as percent conversion of starting material to monoaoylated product.

**PCR from RNA**

cDNA Synthesis—Total RNA (1 μg) was denatured at 75°C for 2 min and then chilled on ice. PCR buffer, 1 mM deoxynucleotide triphosphates, 1 unit/μl of RNasin, 100 pmol of random hexamers, and 10 units/μl of Moloney murine leukemia virus reverse transcriptase was then added to a final volume of 20 μl and incubated at 37°C for 1 h.

PCR—80 μl of PCR buffer containing 50 pmol of each upstream (1 below) and downstream (2 below) primer and 0.06 unit/μl of Taq polymerase (Cetus) was then added. 21 PCR cycles were carried out, each cycle being: 30 s at 94°C, 30 s at 54°C, 30 s at 72°C. Then 50 μl of each sample was removed for Southern blot analysis and the remaining sample underwent a further 5 cycles.

Oct-1: (1) 5'-AATCATAGTATCTCTTCC
(2) 5'-AGCCTTGAACCTCAGCTTTAAG
Oct-2: (1) 5'-GGCCCTCAACCTGAGCTTCAAG
(2) 5'-GATCAGAGATCCTCTCT
Actin: (1) 5'-CCGGGCTGTATTCCCCTCCC
(2) 5'-TCATTGTAGAAGGTTGPG

**RESULTS**

**Oct-1 and Oct-2 Binding with DNA: Mobility Shift Assays**—After culturing Daudi cells with α-IFN for 48 h a clear decrease was observed in the level of the protein-DNA complex formed by Oct-1 and a much larger decrease in the level of the complex formed by Oct-2. This effect was observed in several different extract preparations, two of which are illustrated in Fig. 1, panels a and b. No down-regulation of Oct-1 or Oct-2 binding occurred following treatment with α-IFN for up to 24 h, suggesting that this effect requires relatively prolonged exposure to α-IFN. The specificity of the signals for the octamer-binding proteins was demonstrated by competition experiments using the identical untreated extract and an excess of unlabeled competitor containing the octamer motif or the unrelated binding site for the Sp1 transcription factor (Fig. 1, panel d). In these experiments no effect of α-IFN treatment was observed on the levels of other transcription factors, for example, AP1, TFIIIC, ATF (AP1 is shown in Fig. 1, panel c), which confirmed that this effect was specific to the octamer-binding proteins.

Interferon-α can have many different effects on cell physiology, including the induction of an anti-viral state and the inhibition of cell proliferation. We wished to determine whether the changes in octamer binding were related to any specific change in the cell brought about by α-IFN. To do this we used a variant of the Daudi cell line, DaudiIR which is resistant to the antiproliferative effects of α-IFN but which has functional α-IFN receptors and responds to treatment with α-IFN by the induction of IFN-induced genes (7). Interferon-α treatment of these cells did not result in any reduction in the levels of octamer-binding proteins (Fig. 2, panel A). Similarly no effect of α-IFN treatment on the level of Oct-1 was observed in extracts of HeLa cells (Fig. 2, panel B) which similarly continue to proliferate in the presence of α-IFN and which respond to α-IFN by the induction of IFN-inducible genes (data not shown). As expected these cells did not contain Oct-2 which is found only in B cells and some neuronal cells (13, 31).

The cell cycle of Daudi cells can also be inhibited in G₀/G₁
by culturing with PMA (32). This agent is not known to induce an antiviral state or to induce the same subset of genes as α-IFN (data not shown). As with α-IFN a down-regulation of Oct-1 and, to a lesser extent, of Oct-2 binding was observed after culturing Daudi cells with PMA (Fig. 1).

**Oct-1 and Oct-2 mRNA Levels**—In order to determine whether the effects we observed on Oct-1 and Oct-2 levels in mobility shift assays were mediated by changes in the corresponding mRNAs, we prepared mRNA from α-IFN and PMA Daudi cells. This mRNA was used to prepare cDNA and the cDNA was then used as a template in a PCR with either Oct-1 or Oct-2 specific primers. The results of this experiment (Fig. 3) showed that 48 h of α-IFN treatment produced a large decrease in the level of Oct-2 mRNA in Daudi cells, although no detectable fall in Oct-1 mRNA levels was observed. A smaller but detectable fall in the level of Oct-2 mRNA was also observed after 20 h of α-IFN treatment (data not shown). As expected, no effect on the level of either Oct-1 or Oct-2 mRNAs was observed upon α-IFN treatment of interferon-resistant DaudiIR cells. In contrast, PMA treatment produced a large fall in the Oct-1 mRNA level in Daudi cells without apparently affecting Oct-2 mRNA levels. All samples showed similar levels of mRNA when primers specific for the actin transcript were used in the PCR (data not shown).

These data therefore parallel the results of the mobility shift assays in which α-IFN produced a more dramatic effect on Oct-2 than Oct-1 levels, while PMA had the opposite effect. It may be that only gross changes in mRNA levels can be observed by the PCR method and that smaller changes in the mRNA level of Oct-1 in α-IFN-treated cells and of Oct-2 in PMA-treated cells were not detected in our experiments. However, the data would indicate that at least the most dramatic effects of these agents on the levels of octamer-binding proteins are mediated by corresponding changes at the mRNA level.

**Cell Cycle and DNA Synthesis**—The majority (87% ± 5.6 S.D.) of actively dividing Daudi cells which were not cultured with α-IFN incorporated BrdUrd over a 24-h period (Fig. 4). No significant difference (p > 0.05; Student’s t test) from control values was observed in DNA synthesis in the first 24 h of α-IFN treatment. However, a marked decrease (p < 0.005) in BrdUrd incorporation occurred 24–48 and 48–72 h after the addition of α-IFN. The proportion of cells in the G0/G1 phase of the cell cycle was the same in the control and after 24 h of α-IFN treatment. However, an increase in G0/G1 was observed 48 h (p < 0.01) and 72 h (p < 0.005) after the addition of α-IFN (Fig. 4).

**Gene Expression and Octamer-containing Promoters**—To investigate the effect of α-IFN treatment on octamer-mediated gene expression in Daudi cells, we made use of a series of constructs in which the octamer motif is cloned upstream of the HSV thymidine kinase promoter in the vector pBl2CAT (24). Daudi cells were transfected with these constructs and CAT activity was assayed 48 h after the addition of α-IFN. As shown in Fig. 5 and 6, α-IFN treatment resulted in a 3–5-fold decrease in the activity of each of the octamer-containing promoters (constructs a–d). In contrast no effect of α-IFN treatment was observed on the activity of the parental pBl2CAT vector (Fig. 6, u) or on that of an Rous sarcoma virus-CAT construct (28) (Fig. 5, r) indicating that this effect is specific to octamer-containing promoters. The vector pBl2CAT contains a truncated thymidine kinase pro-

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**Fig. 2.** DNA mobility shift assay using the octamer probe and extracts prepared from DaudiIR cells (A) or HeLa cells (B) without (0) or with 48 h of α-IFN treatment (J).

**Fig. 3.** Polymerase chain reaction using Oct-1 (panel 0-1) or Oct-2 (panel 0-2) specific primers. cDNA was prepared from the mRNA of untreated Daudi cells (tracks 1 and 5), Daudi cells treated with α-IFN (track 2) or PMA (track 6) for 48 h, untreated DaudiIR cells (track 3), or α-IFN-treated DaudiIR cells (track 4). The bands produced by Oct-1 and Oct-2 are indicated. Actin mRNA was amplified as an internal control and was equivalent in each case (not shown).

**Fig. 4.** DNA synthesis, BrdUrd incorporation. Daudi cells were cultured with α-IFN for the time periods shown. DNA synthesis was assayed by BrdUrd incorporation (□) for 0–24, 24–48, and 48–72 h. The percentage of cells in G0/G1 (□) at 0, 24, 48, and 72 h is also shown.

**Fig. 5.** Assay of chloramphenicol acetyltransferase activity in untreated (0) or α-IFN-treated (J) Daudi cells transfected with a Rous sarcoma virus-CAT construct (r) or constructs containing the octamer oligonucleotides a–d (see “Experimental Procedures”) cloned into pBl2CAT (a–d).
Interferon-α Repression of Oct-1 and Oct-2

between Oct-1 and the virion protein Vmw65, which binds to yet to be identified.

the transactivation of the HSV immediate-early genes during lytic infection (37, 38). Transactivation of the immediate-early genes is dependent on the formation of a complex between Oct-1 and the virion protein Vmw65, which binds to the octamer-related TAATGARAT motif in the immediate-early promoters (37, 39). This transactivation event is inhibited in α-IFN-treated cells, resulting in a lack of immediate-early gene expression and a consequent failure of the viral lytic cycle (40, 41). It is possible therefore that the down-regulation of Oct-1 we have observed may be responsible for this effect. In agreement with this idea, three of the constructs (a, b, and c) whose down-regulation we observed in α-IFN-treated cells contain TAATGARAT sequences found in the immediate-early promoters (26, 27) either alone (b) or overlapping an octamer motif (a and c). In addition, a construct in which the HSV immediate-early 3 promoter drives the CAT gene is also down-regulated following α-IFN treatment of Daudi cells (data not shown). However, Oct-1 is not decreased in HeLa or DaudiIR cells after culturing with α-IFN, even though these cells have α-IFN receptors and respond by the induction of IFN-inducible genes (7, 42, 43, and data not shown). Therefore the down-regulation of Oct-1 by α-IFN which occurs in Daudi cells may not in general be part of the antiviral mechanisms induced by α-IFN.

Interferon-α not only regulates gene expression in Daudi cells but also causes the inhibition of cell-cycle progression by arresting cells in G$_1$/G$_2$ (10). However, culturing the DaudiIR or HeLa cell lines with α-IFN does not lead to the inhibition of the cell cycle and these cells continue to proliferate normally. The decline in Oct-1/2 levels brought about by α-IFN occurs in Daudi but not in DaudiIR or HeLa cell lines (Figs. 1 and 2). The timing of this effect correlates with the inhibition of cell cycle progression and DNA synthesis (Fig. 4). In addition, PMA also down-regulates Oct-1/2 levels (Fig. 1). This agent is not known to induce an antiviral state and it does not induce the same subset of genes as α-IFN. However, culturing Daudi cells with PMA causes an inhibition of proliferation and cells accumulate in G$_1$/G$_2$ (32). Therefore, the decline in complex formation between Oct-1/2 and DNA may be part of the mechanism by which α-IFN inhibits DNA replication.

Interestingly, while α-IFN produced a more dramatic decline in Oct-2 levels compared to Oct-1, the reverse effect was observed with a PMA where Oct-1 levels were more reduced. These effects were paralleled at the RNA level where the levels of Oct-2 mRNA in α-IFN-treated cells and of Oct-1 mRNA in PMA-treated cells were greatly reduced compared to those observed in untreated cells. Hence the most dramatic effects of these agents on the levels of octamer-binding protein observed in DNA mobility shift assays are probably produced by a decline in the levels of their corresponding mRNAs rather than, for example, by a change in the DNA binding ability of the protein. Our inability to observe any change in the Oct-1 mRNA level following α-IFN treatment or in the Oct-2 mRNA level following PMA treatment may reflect the inability of our PCR procedure to detect relatively small alterations in mRNA levels. Alternatively, the smaller changes in protein binding observed in these cases may be produced by changes in the translation of pre-existing mRNA or in the DNA binding ability of pre-existing protein without any alteration in mRNA level.

The declining levels of octamer-binding proteins which we have observed could affect cell division either directly or indirectly. An indirect mechanism might involve inhibiting histone H2B synthesis, which is regulated by Oct-1 (12), since cells with artificially lowered levels of this histone have been shown to grow more slowly than normal cells (44). Alternatively, since both Oct-1 and Oct-2 can act as DNA replication factors (18), it is possible that the decline in their abundance directly affects DNA replication. Another octamer-binding factor, Oct-3, has been shown to be required for DNA replication to occur in mouse embryonic cells (45). Therefore,
octamer-binding proteins may play a role not only in viral DNA replication (18) but also in S-phase in mammalian cells. We have shown previously that α-IFN also causes dephosphorylation of the cell cycle control protein p34cdc2 and the retinoblastoma tumor-suppressor protein, Rb (32, 46). Therefore, α-IFN may arrest the cell cycle in G0/G1, and prevent entry into S-phase by affecting two or more processes, the first involving p34cdc2 and Rb proteins, and the second involving the octamer-binding proteins described here.

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