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Interferon-dependent induction of mRNA for the major histocompatibility antigens in human fibroblasts and lymphoblastoid cells

(gene expression/cDNA/(2'-5') oligo(A) synthetase/human leukocyte interferon/human fibroblast interferon)

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ABSTRACT **In human cells treated with interferons, there is an increase in the amount of HLA-A,B,C and β_2-microglobulin exposed on the cell surface. We have used a cloned HLA-A,B,C cDNA probe to demonstrate by molecular hybridization that this effect of interferon is preceded by a large increase in the amount of HLA mRNA in the cell. This effect was found in five different human cell lines, with purified leukocyte and fibroblast interferons. The increase in HLA mRNA is comparable in its kinetics and dose–response to the induction of (2'-5') oligo(A) synthetase mRNA by interferons. Therefore, interferons seem to activate at least two cellular genes which have different biochemical functions.**

Studies on the mechanism by which interferons (IFNs) inhibit virus multiplication have led to the discovery of several enzymes, inhibitors of protein biosynthesis, which are induced in cells exposed to IFNs (for review, see refs. 1–3). An assay for the mRNA of one of these enzymes, (2'-5') oligoadenylate synthetase (synthetase E), was developed and it was shown that IFNs induce, within a few hours, the accumulation of specific synthetase E mRNA in the cytoplasm of treated cells (4). Inhibitors of transcription and translation block the induction of synthetase E (5–8). The same is true of the IFN-induced eukaryotic initiation factor 2 protein kinase (5) and of 5–10 other proteins which can be detected by one- or two-dimensional polyacrylamide gel electrophoresis (9–13). Induction of these proteins by IFN therefore is likely to take place at the gene level.

In addition to the above effects, IFNs also cause complex changes in the plasma membranes of cells (14). These changes alter the electric charge of the cells (15), decrease their motility (16), and influence cell–cell recognition events involved in the immune response (14, 17). Biochemically, these membrane changes affect both the lipids (18) and several surface protein antigens (19, 20). Thus, human IFNs increase the amounts of HLA-A,B,C antigens and of β_2-microglobulin available for interaction with specific antibodies on the surface of human cells (21, 22). These changes are specific because there is no increase in HLA-DR or in many other surface antigens. We wondered whether these membrane effects of IFNs were topical modifications in the exposure of antigens on the cell surface or involved changes in gene expression similar to those found for synthetase E.

To investigate this question we used a cloned HLA cDNA probe, pHLA-1 (23), to study the HLA-A,B,C mRNA levels in IFN-treated cells. Our results show that, in five human cell lines of lymphoblastoid and fibroblastic origins, both leukocyte IFN (IFN-α) and fibroblast IFN (IFN-β) produce a large and rapid increase in HLA-A,B,C mRNA, which often precedes the increase in synthetase E mRNA.

MATERIALS AND METHODS

Cells and IFNs. Human lymphoblastoid cell lines Ramos (24), Daudi (25), Namalva (26), and Chevalier (27) are derived from Burkitt lymphomas. K562 is derived from a chronic myelogenous leukemia (28). Stationary cultures of these cells were grown in RPMI-1640/10% fetal calf serum to 2×10^6 cells per ml. Human foreskin diploid fibroblasts FS11 (29) were grown to confluency in minimal Eagle's medium/10% calf serum/20 mM Hepes, pH 7.5, without CO_2. SV80 cells [simian virus 40-transformed fibroblastoid cells (30)] were grown in Dulbecco's modified Eagle's medium with 10% calf serum in 8% CO_2/92% air. All cultures were grown at 37°C in the presence of 100 units of penicillin and 100 μg of streptomycin per ml. Human IFN-α was purified (31) from Sendai virus-infected chronic myelogenous leukemic cells, obtained from Institut Merieux (Lyon, France). Human IFN-β was prepared as described (29, 32) from poly(rI)·poly(rC)-superinduced FS11 fibroblasts (InterYeda, Rehovot, Israel). The specific activities of these IFNs are given in the legend of Table 1.

Immunological Assays. To measure the surface HLA-A,B,C antigen, the cell cultures were washed twice with RPMI-1640/0.5% bovine serum albumin/0.1% Na azide. Aliquots (2.5×10^5 cells) were mixed with 50 μl of diluted monoclonal antibody W6/32 (33), which recognizes a monomorphic determinant of the HLA-A,B,C chains. After 1 hr at 4°C the cells were washed as above and 10^5 cpm of ^{125}I-labeled protein A was added. After 1 hr at 4°C, the cells were washed and assayed for ^{125}I. For HLA-DR, the monomorphic monoclonal antibody L116 (34) was used; total surface antigens were measured with a rabbit antiserum against WI-38 human fibroblasts. About 10% background binding of ^{125}I without antibodies was subtracted.

HLA mRNA Assay. Total cell RNA was prepared by the LiCl method (35, 36) from $2–4 \times 10^8$ lymphoblastoid cells or 6×10^7 FS11 cells. The cells were washed with serum-free medium and homogenized for 1 min in a Waring Blendor with 20 ml of 3 M LiCl/6 M urea/10 mM Na acetate, pH 5, containing 200 μg of heparin per ml and 0.1% $NaDodSO_4$. After 16 hr at 4°C, the pellet was recovered by centrifugation at $15,000 \times g$ for 30 min, washed twice with 20 ml of 4 M LiCl/8 M urea, and dissolved

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Abbreviations: HLA, major human histocompatibility antigens; synthetase E, (2'-5') oligoadenylate synthetase; IFN, interferon; IFN-α, leukocyte IFN; IFN-β, fibroblast IFN; NaCl/Cit, standard saline citrate.

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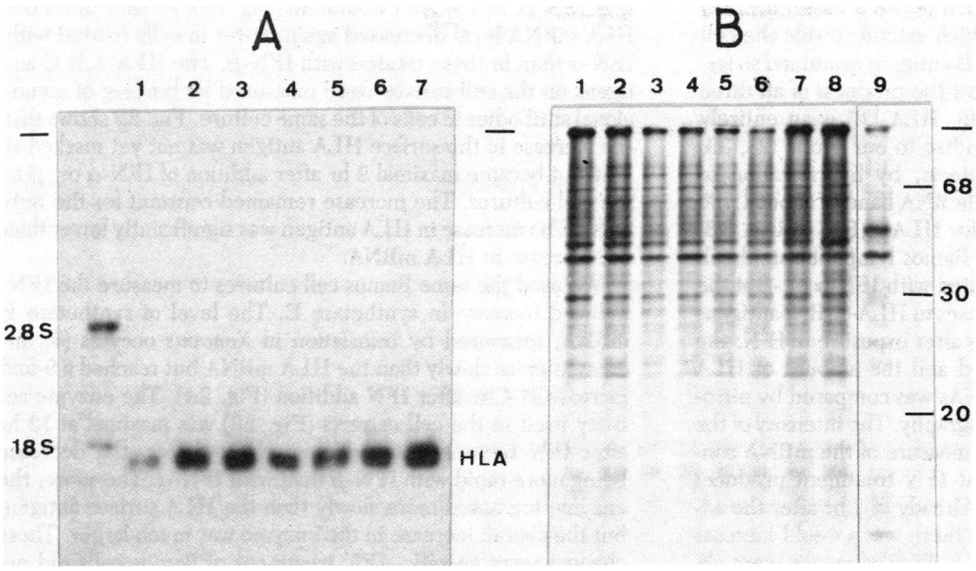

FIG. 1. (*A*) Detection of HLA-A,B,C mRNAs. Ramos cultures were treated with IFN-α (1,000 units/ml) or IFN-β (200 units/ml) for various times. Poly(A)$^+$RNA was isolated, subjected to agarose gel electrophoresis, and, after blotting to nitrocellulose, hybridized to pHLA [^{32}P]DNA. Ribosomal [^{32}P]RNA was used as marker. The autoradiograph is shown. Lanes: 1, no IFN; 2–5, IFN-α for 1, 2, 6, and 12 hr; 6 and 7, IFN-β for 2 and 4 hr. (*B*) Translation of Ramos cell/poly(A)$^+$RNA in reticulocyte lysates. About 0.2–0.3 μg of RNA was translated as described (29) and the products were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Lanes: 1, no IFN; 2–5, IFN-α for 1, 2, 6, and 12 hr; 6–8, IFN-β at 2, 4, and 6 hr; 9, tubulin and actin markers. Right scale, $M_r \times 10^{-3}$.

in 5 ml of 0.1 M Na acetate, pH 5/0.1% NaDodSO$_4$. The RNA was extracted with 2 vol of phenol/chloroform, 1:1 (vol/vol), and then with 1 vol of chloroform/isoamyl alcohol, 24:1, and ethanol precipitated. The RNA was dissolved in 0.5 M NaCl/10 mM Tris·HCl, pH 7.5/0.1% NaDodSO$_4$ and passed three times through an oligo(dT)-cellulose column (P.L. Biochemicals, type 7). After a wash with 0.05 M NaCl in the same buffer, the poly(A)$^+$RNA was eluted in 10 mM Tris·HCl, pH 7.5/0.05% NaDodSO$_4$ and ethanol precipitated. For electrophoresis, 2 μg of poly(A)$^+$RNA was heated to 60°C for 10 min in 20 μl of 50% (vol/vol) formamide/6% (vol/vol) formaldehyde in Mops buffer (20 mM 4-morpholinepropanesulfonic acid, pH 7/5 mM Na acetate/1 mM EDTA). After quick cooling, 6 μl of 50% (vol/vol) glycerol with bromophenol blue was added and the sample was run at 250 V in a 1.2% agarose slab gel with 6% formaldehyde and Mops buffer, until the dye reached 13 cm from the origin. The gel was soaked for 30 min in 20× standard saline citrate (NaCl/Cit; 0.15 M NaCl/0.015 M Na citrate) and blotted with 10× NaCl/Cit onto nitrocellulose presoaked in 10× NaCl/Cit for 20 min (37). After drying for 4–6 hr at 80°C, the nitrocellulose sheet was prehybridized overnight at 42°C with 50% formamide/5× NaCl/Cit/4× Denhardt's solution (38)/0.1% NaDodSO$_4$/0.1% Na pyrophosphate (1 ml/15 cm^2) containing 250 μg of *Escherichia coli* DNA per ml.

Hybridization was carried out for 20 hr at 42°C under the same conditions, but with 10^6 cpm of pHLA[^{32}P]DNA per ml ($2–3 \times 10^8$ cpm/μg) prepared by nick-translation as follows. A mixture of 0.2 μg of pHLA-1 plasmid DNA (23), 80 μM dGTP, 80 μM dTTP, 25 μCi of [^{32}P]dATP and 25 μCi of [^{32}P]dCTP (both 500 Ci/mmol; Amersham; 1 Ci = 3.7×10^{10} becquerels) and DNase I (4 ng/ml) was incubated in 25 μl for 1 min at room temperature; then 2 units of DNA polymerase I was added and the incubation was continued at 14°C until 30% of the radioactivity was incorporated. The reaction was diluted 1:8 and filtered through a Sephadex G-50 column. After hybridization, the blots were washed with 15 mM NaCl/1.5 mM Na citrate/0.1% NaDodSO$_4$ once at 20°C and then three times for 30 min at 50°C, dried, and exposed to Agfa Curix x-ray film with intensifying screens at −70°C.

RESULTS

HLA-A,B,C are transmembrane proteins consisting of a M_r 44,000 polypeptide chain complexed with β_2-microglobulin (for review, see ref. 39). The cloned HLA cDNA probe that we used, pHLA-1, corresponds to the 3′ third of the HLA mRNA (23). This is the region that codes for the carboxy-terminal amino

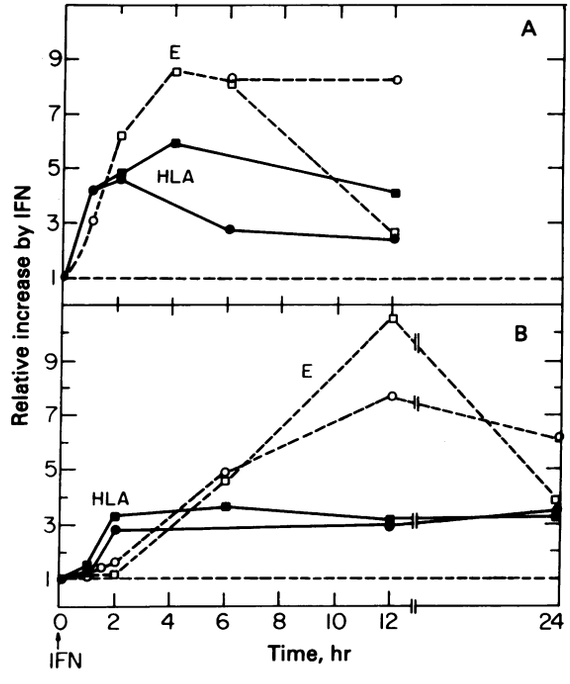

FIG. 2. Kinetics of HLA-mRNA and antigen increase in Ramos cells treated with IFN (●, ○, IFN-α; ■, □, IFN-β). (*A*) The intensity of the HLA mRNA bands from autoradiographs such as in Fig. 1*A* was measured with a Cliniscan scanner. The HLA mRNA levels are shown (solid lines) as a ratio to the level in untreated cells. To measure the synthetase E mRNA (broken lines), groups of *Xenopus laevis* oocytes were injected with 0.5 μg of poly(A)$^+$RNA and the enzyme formed was measured in the oocyte extracts as described (4). Oocytes injected with mRNA from untreated cells gave an enzyme activity of 2,400 cpm [^{32}P](A2′p)$_n$A. The synthetase E mRNA levels in IFN-treated cells are shown relative to this value. Water-injected oocytes gave a background of 260 cpm, which was substracted. (*B*) The HLA-A,B,C surface antigens measured by the binding assay. The results (solid lines) are expressed as a ratio to the radioactivity bound on untreated cells, 3,000 cpm. Synthetase E (broken lines) was measured in extracts prepared as described (4) from 2×10^7 cells with 1 ml of extraction buffer containing Nonidet-P40; 10 μl was used to measure the enzyme activity as described (4). The results are shown relative to the activity in untreated cells [10,000 cpm of [^{32}P](A2′p)$_n$A].

acids of the polypeptide and a portion of the 3′ nontranslated region. This portion of the chain which extends inside the cell appears to be conserved for HLA-A,B antigens examined so far, and the probe probably would detect the products of all three genes—HLA-A, -B, and -C (23, 40). HLA-DR is an entirely different gene and would not hybridize to our probe (23, 39, 40). The pHLA-1 DNA probe detects, by hybridization to poly(A)$^+$RNA of human cells, a single RNA band of about 1,700 nucleotides (Fig. 1*A*), as expected for HLA-A,B,C mRNA (23, 41). In the experiment of Fig. 1*A*, Ramos lymphoblastoid cell cultures HLA-A3, B5 (24) were treated with IFN-α or -β at the concentrations giving optimal increases in HLA-A,B,C antigens on the cell surface. At various times after exposure to IFN, the cellular poly(A)$^+$RNA was extracted and the amount of HLA [^{32}P]cDNA hybridizing to these RNAs was compared by nitrocellulose blot analysis and autoradiography. The intensity of the mRNA band gives an approximate measure of the mRNA concentration, and Fig. 2*A* shows that IFN treatment produces significant increases in this value. Already at 1 hr after the addition of IFN-α to the cell culture, there was a 4-fold increase in the amount of HLA-A,B,C mRNA. Similar results were obtained with total cell RNA not selected for poly(A) sequences (not shown).

In Ramos cells, the largest increase of HLA mRNA was seen with IFN-β, at 4 hr after treatment (Fig. 2*A*). At later times the HLA mRNA level decreased again, faster in cells treated with IFN-α than in those treated with IFN-β. The HLA-A,B,C antigens on the cell surface were measured by binding of monoclonal antibodies to cells of the same culture. Fig. 2*B* shows that the increase in the surface HLA antigen was not yet marked at 1 hr but became maximal 2 hr after addition of IFN-α or -β to the cell cultures. The increase remained constant for the next 24 hr. The increase in HLA antigen was significantly lower than the increase in HLA mRNA.

We used the same Ramos cell cultures to measure the IFN-induced increase in synthetase E. The level of synthetase E mRNA, measured by translation in *Xenopus* oocytes (4), increased more slowly than the HLA mRNA but reached a 9-fold increase at 4 hr after IFN addition (Fig. 2*A*). The enzyme activity itself in the cell extracts (Fig. 2*B*) was maximal at 12 hr after IFN treatment and decreased thereafter, the decrease being more rapid with IFN-β than with IFN-α. Therefore, the enzyme increased more slowly than the HLA surface antigen, but the overall increase in the enzyme was much larger. These changes were specific. IFN treatment of Ramos cells did not increase HLA-DR or other surface antigens (Table 1). Furthermore, translation of the poly(A)$^+$mRNAs from Ramos lymphoid cells in a reticulocyte lysate and analysis of the translation

Table 1. HLA-A,B,C mRNA and surface antigens in various human cells treated with IFN

	IFN treatment			HLA-A,B,C mRNA cDNA hybridized		HLA-A,B,C antigen		HLA-DR antigen bound to antibody		Total surface antigens	
Cells	Type	Units/ml	Time, hr	Ratio	Arbitrary scan units	Ratio	cpm	Ratio	cpm	Ratio	cpm
Ramos	None			1.0	1.6	1.0	13,800	1.0	5,400	1.0	3,000
	α	1,000	12	6.2		—		—		1.0	
	α	1,000	24	2.4		2.4		0.94		—	
	β	200	12	10.8		—		—		1.0	
	β	200	24	6.2		2.5		0.98		—	
Chevalier	None			1.0	0.7	1.0	2,500			1.0	1,500
	α	400	24	2.0		4.0				1.3	
Namalva	None			1.0	1.5	1.0	11,000	1.0	6,000		
	α	1,000	24	1.1		1.0		0.83			
	β	400	24	1.2		0.82		1.2			
Daudi	None			1.0	1.7		ND				
	α	2,000	24	2.0			ND				
	β	200	24	5.0			ND				
K562	None				ND		ND			1.0	3,000
	α	1,000	24		ND		ND			1.0	
	β	2,000	24		ND		ND			1.0	
FS11	None			1.0	3.0	1.0	22,000			1.0	15,000
	α	1,000	19	2.0		2.2				1.1	
	β	200	19	2.8		2.0				1.0	
SV80	None			1.0	2.5	1.0	4,000			1.0	2,000
	α	1,000	16	6.7		3.0				1.0	
	β	200	16	8.2		4.5				1.2	

For the experiments with Ramos, Chevalier, and Daudi lymphoid cell lines, a pure preparation of IFN-α subspecies β_3 (31)—IFN-α (B) (47)—was used. This IFN was purified by repeated high-pressure liquid chromatography (31). Its specific activity was 5×10^8 units/mg of protein on human cells and 2×10^8 units/mg on bovine cells; it was pure on $NaDodSO_4$/polyacrylamide gel electrophoresis. For the other experiments, less purified unfractionated mixtures of IFN-α species were used. Fibroblast IFN-β was purified (32) to $>10^8$ units/mg of protein. For SV80 cells, the poly(A)$^+$RNA was extracted from cytoplasmic extracts prepared as described (29) with Nonidet-P40 from 6×10^8 cells. The results are given relative to the values in untreated cells; control absolute values are also shown to allow comparison of the different cell cultures. ND, not detectable.

products by polyacrylamide gel electrophoresis revealed no gross variations in mRNA activity after IFN treatment (Fig. 1*B*) and only limited increases or decreases in a few individual mRNAs.

IFN-dependent increases in HLA-A,B,C mRNA and surface antigen levels were observed also in several other human cell lines (Table 1). We first compared Ramos lymphoid cells to three other Burkitt lymphoid lines. In Chevalier lymphoid cells, IFN increased both HLA-A,B,C mRNA and surface antigens. In contrast, in Namalva lymphoid cell cultures there was no increase in the HLA antigens or mRNA with either IFN-α or -β. The growth of these cells is not inhibited by IFN (42), but synthetase E is induced by IFN in Namalva cells (43). Finally, Daudi lymphoid cells were also studied because these cells lack β_2-microglobulin (44, 45) and hence have no HLA-A,B,C on their cell surface. Nevertheless, Daudi lymphoid cells contain HLA mRNA (Table 1) and both IFN-α and -β were able to increase the HLA mRNA, indicating that the IFN effect does not require the presence of the HLA complex on the cell surface. We next studied the cell line K562 because it also is deficient in surface HLA, but for a different reason. K562 cells are pre-erythroid cells which produce embryonic hemoglobin (46); they have no detectable HLA mRNA (Table 1). In these cells, IFN could not turn on detectable expression of the HLA genes, at least not in the 24-hour period studied. Table 1 also shows the results obtained with two human fibroblast cell lines. In diploid foreskin fibroblasts FS11, both IFN-α and -β increased by a factor of 2–3 the amounts of HLA mRNA and antigen expressed. Similarly, SV80 cells responded extremely well to IFN, showing up to 8-fold increases in HLA mRNA. The kinetics of the IFN-β effect on SV80 are shown in Fig. 3*A*.

In several of the experiments of Table 1 we used an extensively purified preparation of one of the IFN-α subspecies called IFN-$\alpha(\beta_3)$ (31) or IFN-α(B) (47), and we used also fibroblast IFN-β_1 highly purified on blue Sepharose (32). The induction of HLA mRNA is therefore a genuine effect of IFN. Fig. 3*B* shows that low concentrations of IFN are sufficient to induce the HLA. In this experiment using SV80 cells, induction of HLA antigens was more sensitive to IFN-β than was induction of synthetase E, although the amplitude of the variations in HLA was much smaller than the amplitude of the variation in the enzyme. The same was found in Ramos cells and with both IFN-α and IFN-β. The total surface antigens, measured with rabbit anti-human cell antibodies, did not show any significant variation even at high IFN concentrations (Fig. 3; Table 1).

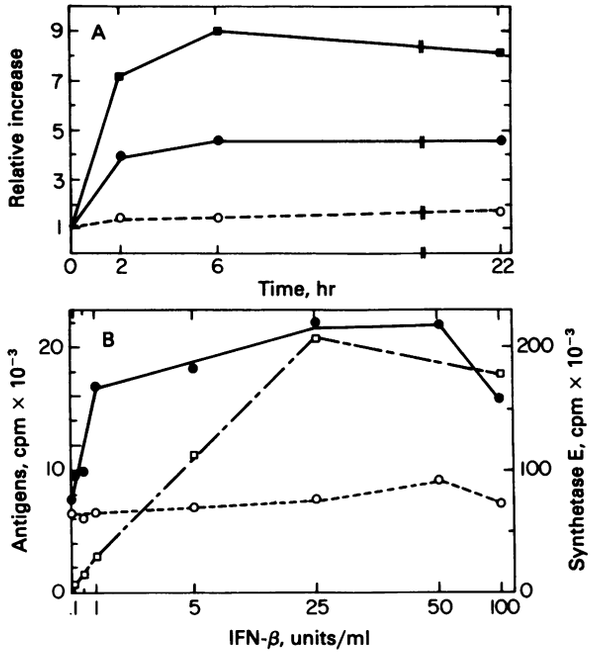

FIG. 3. HLA-A,B,C mRNA increase in SV80 cells treated with IFN. (*A*) Cultures of 2×10^8 cells were used to measure HLA-A,B,C mRNA (■) and surface antigen (●) or total surface antigens (○) at different times after treatment with IFN-β (100 units/ml). The ratios of the values in treated and untreated cells are shown. (*B*) Cultures were treated for 16 hr with different concentrations of IFN-β. The measured value of HLA-A,B,C (●) and total surface antigens (○) are expressed in specific cpm above the background; synthetase E activity (□) was measured as in Fig. 2, and is expressed in cpm of $[^{32}P](A2'p)_n$ A.

DISCUSSION

The main conclusion of our work is that the increase in the surface HLA-A,B,C and β_2-microglobulin produced by IFNs, is preceded by an increase in the amount of HLA mRNA in the cell. This increase is rapid and occurs within 1 hr of IFN treatment. We have not demonstrated directly that this effect is due to an increased activity of the HLA-A,B,C genes. However, this is the most likely interpretation because there was no obvious change in the stability of the HLA mRNA after IFN treatment, and there was no gross variation in the total mRNA populations of the cells. We recently found that the β_2-microglobulin mRNA is also increased by IFN treatment. In preliminary experiments, we found also that, in Ramos lymphoid cells, the amount of tubulin mRNA was markedly increased after IFN treatment but returned to the normal within 6 hr (unpublished data). Together with our previous demonstration that IFN induces synthetase E mRNA (4), the present data strongly support the idea that the major effect of IFN on cells is to control the expression of specific genes.

The significance of the increase in HLA expression is still unclear. An interesting finding is that in some cases this effect of IFNs can be dissociated from the induction of synthetase E. Thus, as shown here, IFN does not induce the HLA antigen or its mRNA in Namalva lymphoid cells, but it does induce synthetase E (43). Conversely, we found in other experiments that the immune IFN, IFN-γ, induces the HLA-A,B,C antigens much more than it does synthetase E; a more detailed comparison of the effects of the various human IFN species and subspecies on different cell lines is presented elsewhere. Many lines of evidence link the induction of synthetase E with the inhibition of protein synthesis related to IFN's antiviral and antigrowth effects (1, 48). Although the increase in HLA-A,B,C expression may also be related to cell growth (49), its major function is probably in the immunoregulatory actions of IFNs (14), such as tumor rejection. In any event, comparison between the various genes turned on by IFNs should become useful for elucidating the molecular mechanism of IFN action.

Note Added in Proof. We recently confirmed the HLA mRNA induction with another HLA-B cDNA probe (50) kindly given to us by S. M. Weissman. An increase in β_2-microglobulin mRNA was similarly demonstrated with cloned cDNA from K. Itakura (51).

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