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Production of a Monoclonal Antibody Directed against an Interferon-Induced 56,000-Dalton Protein and Its Use in the Study of This Protein

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Interferon (IFN) treatment of cells induces the synthesis of several new proteins. A hybridoma cell line producing monoclonal antibody to the IFN-induced 56,000-dalton protein has been developed. The IFN-induced 56,000-dalton protein is synthesized by a variety of different cells and in response to IFN-α, IFN-β, and IFN-γ. The induction of this protein is dependent on de novo RNA synthesis, since its induction is inhibited if actinomycin D and the IFNs are added to the cells simultaneously. Labeling of IFN-treated cells at 4-h intervals at various times after the addition of the IFNs reveals that the synthesis of the 56,000-dalton protein in IFN-α-treated cells peaks within 12 h after the addition of the IFN and is no longer enhanced 20 h after exposure to the IFN. In contrast, IFN-γ-treated cells continue to show an enhanced synthesis of this IFN-induced protein even after 20 h of exposure to the IFN. Thus, the synthesis of the IFN-induced 56,000-dalton protein is regulated differently by the different IFNs. When cells are treated with IFN-α or IFN-γ in the presence of cycloheximide, and actinomycin D is added prior to the removal of the cycloheximide, the cells produce the IFN-induced 56,000-dalton protein and develop an antiviral state in response to both IFN-α and IFN-γ. These results demonstrate that the synthesis of the 56,000-dalton protein is not dependent on the synthesis of an intermediary protein and that the establishment of an antiviral state occurs in the absence of multiple transcriptional events.

The interferons (IFNs) are a family of protein molecules which, after binding to cell surface receptors of responsive cells, are capable of mediating an antiviral state (19, 31), an antiproliferative effect (34, 36), and a variety of immune responses (14). Two classes of IFNs, IFN-α and IFN-β, have been found to bind to the same cell surface receptor, whereas IFN-γ binds to a different receptor (4, 10, 20, 29). The establishment of the antiviral effect by the IFNs requires de novo synthesis of cellular mRNAs and proteins, since the development of an antiviral state can be blocked by inhibitors of transcription and translation (12, 21, 35). Much recent research on the mechanism of action of the IFNs has focused on the induction of the genes and protein products in response to IFN treatment. To date, IFN treatment has been shown to result in elevated levels of at least two enzyme activities and several new mRNAs and proteins (19, 30). Several investigators have examined the pattern of proteins induced by the different IFNs and report that IFN-α and IFN-β induce proteins with similar characteristics (27, 39). IFN-γ treatment of cells, on the other hand, induces some of the same proteins and some unique proteins and fails to induce some of the proteins induced in response to IFN-α and IFN-β (27, 39). In addition to the differences observed in the pattern of proteins synthesized in cells treated with the different IFNs, various investigators have demonstrated that the kinetics and regulation of the development of the antiviral state in response to IFN-α and IFN-β are different from those observed in IFN-γ-treated cells (8, 9).

Thus far, many of the comparisons among the proteins induced by the different IFNs have been made by determining their molecular weights and their migration on two-dimensional gels. Various investigators have reported that proteins with similar molecular weights and isoelectric points are synthesized in cells treated with the different IFNs (27, 39). However, the relatedness of these proteins has not been definitively established. To allow for a further characterization, comparison, and study of the proteins induced by the IFNs, we and others (2, 5, 7, 13, 18, 24) have begun developing antibodies capable of recognizing the IFN-induced proteins. We report here on the development of a monoclonal antibody capable of recognizing the 56,000-dalton protein induced in IFN-α-, IFN-β-, and IFN-γ-treated cells and on the use of this antibody to study the regulation of the synthesis of this protein.

MATERIALS AND METHODS

Materials. [^{35}S]methionine was purchased from E. I. Dupont/New England Nuclear Corp., North Billerica, Mass. GM2767 cells were obtained from the Human Genetic Mutant Cell Repository, Camden, N.J. FS_4 cells were kindly provided by J. Vilcek. WI-38 and Daudi cells were obtained from the American Type Culture Collection, Rockville, Md. Natural human IFN-α (specific activity, $>10^8$ reference units (U)/mg of protein) was purified by using an NK-2 monoclonal antibody column (Celltech, Slough, United Kingdom). Recombinant human IFN-β and recombinant human IFN-γ were purchased from Triton Biosciences, Alameda, Calif., and Amgen Biologicals, Thousand Oaks, Calif., respectively. All IFN amounts are expressed in international reference units (U).

Preparation of [^{35}S]methionine-labeled extracts from untreated and IFN-treated cells. Cells were grown in either Eagle minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum or RPMI 1640 with 10% heat-inactivated fetal bovine serum. The cells were labeled with [^{35}S]methionine in one of the above media containing

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10% of the regular concentration of methionine (labeling media) in either the presence or absence of the IFNs as indicated in the figure legends. Following the labeling periods, the cells were washed with phosphate-buffered saline and lysed in a buffer containing 10 mM Tris hydrochloride (pH 7.4), 50 mM KCl, 5 mM $MgCl_2$, and 0.2% Triton X-100. After 5 min the lysed cells were harvested, and the lysate was centrifuged at 1,500 × *g* for 6 min. The resulting supernatant was centrifuged at 10,000 × *g* for 10 min, and the supernatant (S-10) was collected. Hot trichloroacetic acid-precipitable counts were determined for all S-10 preparations.

Analysis by SDS-PAGE and isoelectric focusing. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 18-cm 12% acrylamide slab gels as described (16, 26). Isoelectric focusing was performed with slab gels as described (25). The resulting gels were processed for autoradiography or fluorography as necessary.

Preparation of antigen for immunization. ^{35}S-labeled extracts of untreated cells or cells treated with a combination of 300 U of IFN-α per ml and 300 U of IFN-γ per ml were fractionated by SDS-PAGE. The IFN-induced 56,000-dalton protein was localized and electroeluted from the gel in a dialysis membrane at 40 mA in 10 mM Tris-acetate (pH 8.8) buffer for 1 h.

Immunization of mice, fusion protocol, and antibody screening methodology. Three BALB/c mice were each injected intraperitoneally at 2-week intervals with 3 μg of the electroeluted 56,000-dalton protein. After 10 immunizations, one of the animals was left for 5-weeks and then injected intraperitoneally with 3 μg of the electroeluted protein. At 4 days later, the splenocytes of this mouse were fused with the P_3U_1 mouse plasmacytoma cell line. The cells were then plated in 96-well plates (Becton Dickinson Labware, Oxnard, Calif.) and allowed to incubate until colonies appeared in the wells. The media in wells in which colonies could be detected were harvested and tested in an enzyme-linked immunosorbent assay for their ability to recognize the 56,000-dalton protein. For the enzyme-linked immunosorbent assay, Immulon 2 plates (Dynatech Laboratories, Inc., Alexandria, Va.) were each coated with 30 ng of the electroeluted 56,000-dalton protein, and the presence of antibody to the 56,000-dalton protein was determined by using an anti-mouse immunoglobulin test kit (Vector Laboratories, Inc., Burlingame, Calif.). Cells present in wells whose culture media tested positive in the enzyme-linked immunosorbent assay were subcloned twice and then tested for their ability to immunoprecipitate the IFN-induced 56,000-dalton proteins. One hybridoma (no. 115) whose culture medium was capable of immunoprecipitating the IFN-induced 56,000-dalton protein was isolated.

Immunoprecipitation of the IFN-induced 56,000-dalton protein. ^{35}S-labeled extracts containing equal amounts of radioactivity were mixed with 200 μl of culture medium in which hybridoma 115 was grown and then allowed to incubate for 30 min. To these samples, 20 μl of anti-mouse immunoglobulin prepared in rabbits (Accurate Corp., Westbury, N.Y.) was added, and the mixture was allowed to incubate for 30 min. The immune complex was precipitated by adding 100 μl of a 10% solution of *Staphylococcus aureus* Cowan 1 fixed with Formalin (Pansorbin; Calbiochem-Behring, La Jolla, Calif.). Following a 30-min incubation period, the mixture was gently layered onto 500 μl of a buffer containing 1 M sucrose, 0.5 M NaCl, 0.5% Nonidet P-40, and 1% polyoxyethylene-20-cetyl ether (Brij 58) and then centrifuged for 2 min at 10,000 × *g*. The supernatant was then removed, and the pellet was washed once with the sucrose buffer and then four times with a buffer containing 0.02 M KPO_4 (pH 6.8), 0.15 M NaCl, 0.5% Nonidet P-40, 1% polyoxyethylene-20-cetyl ether, and 5 mg of bovine serum albumin per ml. Following the last wash, the pellets were harvested by centrifugation, and the bound proteins were eluted in either a buffer containing 2.5% SDS and 4.2% β-mercaptoethanol (for SDS-PAGE) or 9 M urea and 5% β-mercaptoethanol (for isoelectric focusing). To ensure quantitative immunoprecipitations, the amounts of antibodies and *S. aureus* used were in excess of that required to obtain complete precipitation of the induced protein. The eluted proteins were analyzed by either SDS-PAGE or isoelectric focusing.

Infection with vesicular stomatitis virus and determination of virus yield. Cultures of FS_4 cells were challenged with vesicular stomatitis virus at a multiplicity of 20 PFU/cell. Virus adsorption was allowed to take place for 1 h at 37°C, and then the cells were washed and allowed to incubate at 37°C for 18 h. The cultures were then frozen and thawed three times, and the virus yields were determined as described (26).

RESULTS

We have reported that the treatment of human fibroblast cells with human IFN-α or human IFN-β induces the synthesis of 88,000-, 80,000-, 67,000-, and 56,000-dalton proteins and that the treatment of these cells with human IFN-γ induces the synthesis of 88,000-, 67,000-, and 56,000-dalton proteins (27) (see also Fig. 1). In addition, we have recently observed the enhanced synthesis of a 42,000-dalton protein in IFN-γ-treated cells (Fig. 1). The relatedness of the proteins with similar molecular masses that are induced by the different IFNs is currently being investigated.

To study the IFN-induced 56,000-dalton protein and to determine whether the IFN-α-, IFN-β-, and IFN-γ-induced 56,000-dalton proteins are related, we developed a hybridoma producing a monoclonal antibody capable of recognizing the IFN-induced 56,000-dalton protein. An immunoprecipitation with this monoclonal antibody was performed on extracts of GM2767 cells prepared from either untreated cells or cells treated with either 300 U of IFN-α per ml or 300 U of IFN-γ per ml. This antibody is capable of specifically immunoprecipitating the IFN-induced 56,000-dalton protein from IFN-α- and IFN-γ-treated cells (Fig. 1). Since 300 U of the IFNs per ml was found to be a saturating dose with regard to the induction of the antiviral effect and the induction of the 56,000-dalton protein, this dose of IFN was used in this and all subsequent analyses. The recognition of the induced protein by the monoclonal antibody clearly demonstrates the antigenic relatedness between the 56,000-dalton proteins induced by IFN-α and IFN-γ. Since the above experiment was performed with GM2767 cells, which are trisomic for chromosome 21 and thereby express a greater sensitivity to the IFNs (33), we performed immunoprecipitations on extracts prepared from a variety of other IFN-treated cell lines to determine whether other cells also respond to the IFNs by inducing the synthesis of this protein. Treatment of WI-38 and FS_4 cells with IFN-α or IFN-γ induces the synthesis of the 56,000-dalton protein (Fig. 2). Daudi cells, on the other hand, which have been shown to be resistant to the effect of IFN-γ (28, 37), produce the 56,000-dalton protein in response to IFN-α but not IFN-γ. To avoid any confusion that may arise from studies undertaken with a cell line trisomic for chromosome 21, subsequent analyses were performed with the FS_4 cell line,

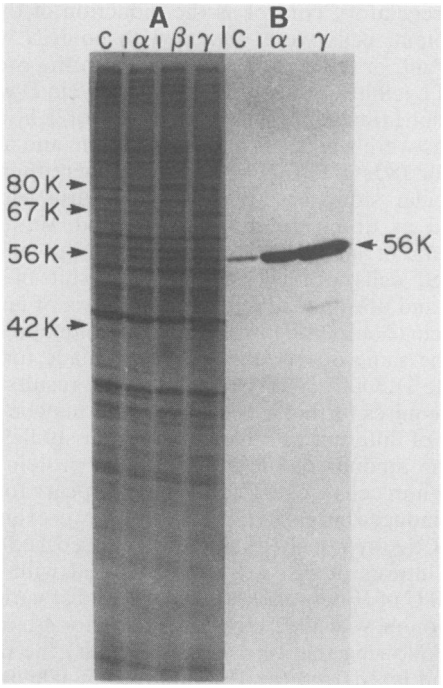

FIG. 1. Induction of proteins in IFN-treated cells and immunoprecipitation of the IFN-induced 56,000-dalton protein. (A) Cultures of GM2767 cells were incubated in labeling media containing 20 μCi of [^{35}S]methionine per ml with either no IFN (lane C), 300 U of IFN-α per ml (lane α), 300 U of IFN-β per ml (lane β), or 300 U of IFN-γ per ml (lane γ). Following an 18-h incubation period, the cells were lysed and extracts were prepared and analyzed by SDS-PAGE as described in Materials and Methods. (B) The 56,000-dalton protein was precipitated from extracts of untreated (lane C), IFN-α (lane α)-treated, and IFN-γ (lane γ)-treated cells containing equal amounts of radioactivity, as described in Materials and Methods. To calculate the molecular masses of the IFN-induced proteins, we ran the following proteins in parallel as molecular mass markers: phosphorylase *b* (94,000 daltons), bovine serum albumin (67,000 daltons), ovalbumin (43,000 daltons), α-chymotrypsinogen (25,700 daltons), and β-lactoglobulin (18,400 daltons). The IFN-induced 80,000-dalton (80K), 67,000-dalton (67K), 56,000-dalton (56K), and 42,000-dalton (42K) proteins are indicated by arrows.

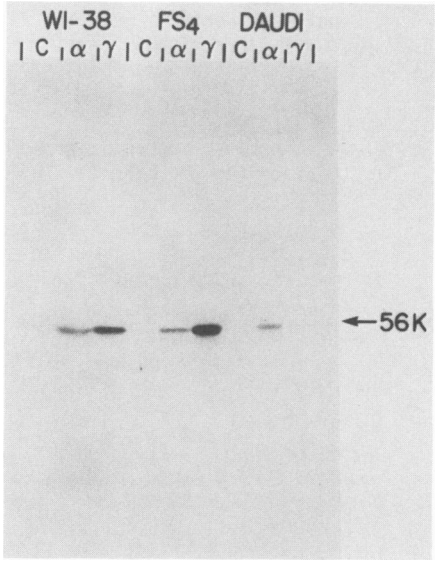

FIG. 2. Immunoprecipitation of the IFN-induced 56,000-dalton protein from various cell lines. Cultures of WI-38, FS_4, and Daudi cells were incubated for 18 h in labeling media containing 20 μCi of [^{35}S]methionine per ml and no IFN (lanes C), 300 U of IFN-α per ml (lanes α), or 300 U of IFN-γ per ml (lanes γ). The 56,000-dalton protein (56K) was immunoprecipitated as described in the legend to Fig. 1 and Materials and Methods.

which is a normal human diploid fibroblast cell. Isoelectric focusing of the immunoprecipitated 56,000-dalton protein produced in FS_4 cells treated with IFN-α, IFN-β, or IFN-γ reveals that the IFN-induced 56,000-dalton protein exhibits microheterogeneity with isoelectric points between 6.2 and 6.7 (Fig. 3).

In further studies with the FS_4 cell line, we observed that the induction of the 56,000-dalton protein is dependent on de novo RNA synthesis, since its synthesis is inhibited by actinomycin D if the actimonycin D is added at the same time as the IFNs (Fig. 4).

Dianzani et al. reported in 1980 that cells treated with IFN-α in the presence of an inhibitor of protein synthesis develop an antiviral state upon removal of the inhibitor (9). IFN-γ-treated cells, however, were reported to be unable to develop an antiviral state under these incubation conditions (9). These results suggest that the establishment of an antiviral effect in IFN-γ-treated cells is dependent on the synthesis of an intermediary protein, which in turn induces the synthesis of proteins that are responsible for the development of the antiviral effect. In IFN-α-treated cells, on the other hand, the establishment of an antiviral effect does not

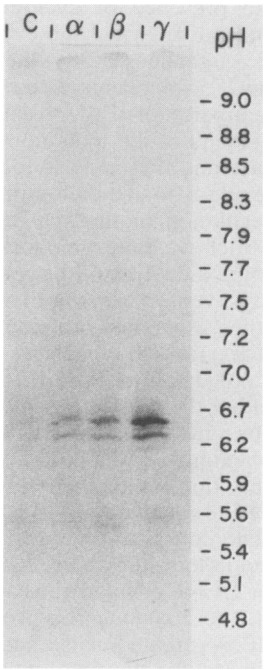

FIG. 3. Isoelectric focusing of the IFN-induced 56,000-dalton protein. Cultures of FS_4 cells were incubated in labeling media for 18 h with 20 μCi of [^{35}S]methionine per ml in the presence of no IFN (lane C), 300 U of IFN-α per ml (lane α), 300 U of IFN-β per ml (lane β), or 300 U of IFN-γ per ml (lane γ). The 56,000-dalton protein was then immunoprecipitated from extracts prepared from these cells and analyzed by isoelectric focusing in vertical slab gels, as described previously (25). The pH gradient of the gel was measured by using a Corning combination pH electrode.

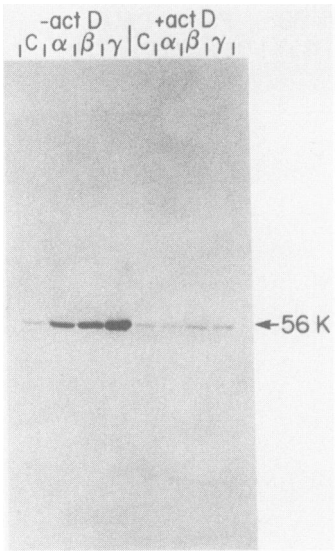

FIG. 4. Effect of actinomycin D on the synthesis of the IFN-induced 56,000-dalton protein. Cultures of FS_4 cells were incubated in labeling media for 18 h with 20 μCi of [^{35}S]methionine per ml with no IFN (lanes C) or 300 U of IFN-α per ml (lanes α), 300 U of IFN-β per ml (lanes β), or 300 U of IFN-γ per ml (lanes γ) and in either the presence (+act D) or absence (−act D) of 2 μg of actinomycin D per ml. Extracts prepared from these cells were analyzed by immunoprecipitation for the presence of the 56,000-dalton (56K) protein.

require the synthesis of an intermediary protein. This study was expanded by others, who reported that cells incubated with IFN-γ in the presence of cycloheximide and then washed free of both the IFN and the cycloheximide fail to synthesize the 2′,5′-oligoadenylate synthetase (1), as well as the mRNAs for the 2′,5′-oligoadenylate synthetase (11). On the other hand, incubation of IFN-α-treated cells under the above conditions results in the induction of the 2′,5′-oligoadenylate synthetase and the mRNAs for the 2′,5′-oligoadenylate synthetase and a 56,000-dalton protein (1, 11).

To study the regulation of the synthesis of the 56,000-dalton protein to which we have a monoclonal antibody, we examined whether the induction of the 56,000-dalton protein occurs as a primary event in response to IFN treatment or whether the synthesis of this protein first requires the synthesis of another protein which in turn induces the synthesis of the 56,000-dalton protein. Cells were treated with the IFNs in either the presence or absence of cycloheximide (50 μg/ml) for 5 h. Actinomycin D (2 μg/ml) was then added to the cycloheximide-containing flasks for 1 h to block subsequent transcription, and then all the flasks were washed and allowed to incubate for 18 h in the presence of [^{35}S]methionine. The concentrations of cycloheximide and actinomycin D used in this study were found to be sufficient to block more than 95% of cellular translation and transcription, respectively. The 56,000-dalton protein is induced in both IFN-α- and IFN-γ-treated cells under these conditions (Fig. 5), thus demonstrating that the synthesis of the 56,000-dalton protein occurs as a direct result of IFN treatment and does not require the synthesis of an intermediary protein. The induction of this protein in IFN-γ-treated cells under these conditions is somewhat surprising in light of the previous reports (1, 11).

To further examine the regulation involved in the responsiveness of cells to IFN and to determine whether the induction of an antiviral state in IFN-treated cells is under the same regulatory control as the induction of the 56,000-dalton protein, cells were treated with no IFN, 300 U of IFN-α per ml, or 300 U of IFN-γ per ml in the presence or absence of cycloheximide for 5 h. Actinomycin D was added to the cycloheximide-containing cultures for 1 h, and all of the cultures were washed with fresh medium and allowed to incubate for 18 h at 37°C. These cultures were then infected with vesicular stomatitis virus, and the virus yields were determined as described in Materials and Methods. The development of an antiviral effect in IFN-α- and IFN-γ-treated FS_4 cells occurs as a direct result of the IFN treatment and does not require the synthesis of an intermediary protein (Table 1). This result is not unique to FS_4 cells, because the same observation has been made for GM2767 cells (Table 1). Our inability to confirm the results of others (1, 9, 11) requires further investigation, but may be the result of the use of different cell lines in different studies.

Since the amount of the 56,000-dalton protein accumulated in 24 h in cells treated with IFN-γ appears to be larger than that induced in IFN-α-treated cells, we examined the kinetics of the synthesis of the IFN-induced 56,000-dalton protein. Cultures of FS_4 cells were treated with either no IFN or 300 U of IFN-α or IFN-γ per ml, and at various times [^{35}S]methionine was added to the cultures for 4-h incubation periods. Following the 4-h labeling periods, the cells were washed and lysed, and the IFN-induced 56,000-dalton protein was immunoprecipitated. The synthesis of the 56,000-dalton protein was detected within the first 4 h after treatment with either IFN (Fig. 6). In IFN-α-treated cells, the synthesis of the 56,000-dalton protein peaked between 4 and 12 h following IFN treatment, and the amount of the protein being synthesized by the cells 20 h after the initiation of the IFN treatment was comparable to that being synthesized by

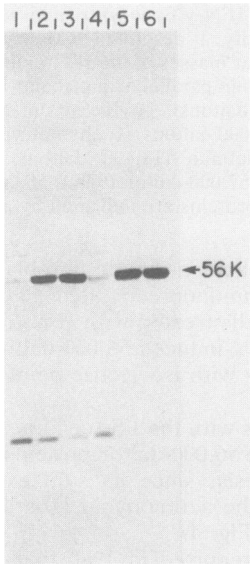

FIG. 5. Induction of the 56,000-dalton (56K) protein does not require synthesis of an intermediary protein. Cultures of FS_4 cells were incubated for 5 h in either the absence of IFN (lanes 1 and 4) or the presence of 300 U of either IFN-α (lanes 2 and 5) or IFN-γ (lanes 3 and 6) per ml and in the presence (lanes 4 to 6) or absence (lanes 1 to 3) of 50 μg of cycloheximide per ml. Actinomycin D was then added to the cycloheximide-containing cultures, and after 1 h, all of the cultures were washed and incubated for 18 h at 37°C in labeling medium containing [^{35}S]methionine (20 μCi/ml). Immunoprecipitations were performed on extracts prepared from these cultures as described in Materials and Methods.

TABLE 1. Study of the regulation of induction of the antiviral state in FS_4 and GM2767 cells

Cell line	Treatment	Incubation[a]	Virus yield (PFU/culture)
FS_4	None	−	3.6×10^7
	IFN-α	−	2.7×10^5
	IFN-γ	−	1.3×10^6
	None	+	1.0×10^7
	IFN-α	+	1.6×10^4
	IFN-γ	+	6.6×10^5
GM2767	None	−	5.7×10^6
	IFN-α	−	7.5×10^2
	IFN-γ	−	5.4×10^4
	None	+	1.0×10^6
	IFN-α	+	5.4×10^2
	IFN-γ	+	2.4×10^4

[a] Incubation under conditions in which only a single transcriptional event can occur.

untreated cultures. IFN-γ-treated cells, on the other hand, continued to show an enhanced synthesis of the IFN-induced 56,000-dalton protein even after 20 h of IFN treatment.

DISCUSSION

IFN treatment of cells has been shown to result in the enhanced synthesis of several new proteins and mRNAs (3, 6, 7, 13, 17, 19, 22, 23, 30, 31, 38). To date, most of the analyses of the IFN-induced proteins have been done by fractionation of cellular extracts by either one- or two-dimensional gel electrophoresis. To study the synthesis of the IFN-induced proteins, we began by developing a monoclonal antibody directed against the IFN-induced 56,000-dalton protein. Using this monoclonal antibody, we have been able to do careful analyses of the synthesis of this protein and to clearly demonstrate that the 56,000-dalton proteins observed to be synthesized in IFN-α-, IFN-β-, and IFN-γ-treated cells are antigenically related and have not only similar molecular masses, but also similar isoelectric points.

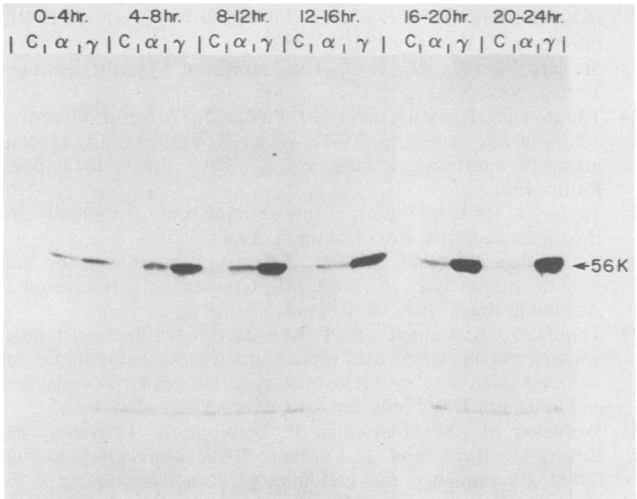

FIG. 6. Kinetics of accumulation of the IFN-induced 56,000-dalton (56K) protein. FS_4 cells were incubated in labeling media in either the absence (lanes C) or presence of 300 U of IFN-α per ml (lanes α) or 300 U of IFN-γ per ml (lanes γ). At 4-h intervals for a period of 24 h, sets of these cultures were incubated with 20 μCi of [^{35}S]methionine per ml for 4 h. At the end of each 4-h interval, the cells were washed, extracts were prepared, and immunoprecipitations were performed as described in Materials and Methods.

Analysis of the kinetics of the synthesis of the IFN-induced 56,000-dalton protein reveals that the synthesis of this protein is regulated differently in cells treated with either IFN-α or IFN-γ. In IFN-α-treated cells, the synthesis of the 56,000-dalton protein is transitory, with the IFN-treated cells no longer exhibiting an enhanced synthesis of this protein after 20 h of exposure to the IFN (Fig. 6). IFN-γ-treated cells, on the other hand, continue to synthesize the 56,000-dalton protein even after 20 h of exposure to the IFN (Fig. 6). Similar results were observed by Sen and Rubin (32) in an SDS-PAGE examination of cell extracts prepared from IFN-α- and IFN-γ-treated cells.

We have demonstrated that the synthesis of the 56,000-dalton protein, as well as the development of the antiviral state in both IFN-α- and IFN-γ-treated cells, occurs as a result of a single transcriptional event and does not require the synthesis of an intermediary protein. The results we have obtained are in contrast to the reports of others (1, 9, 11), who have demonstrated that in IFN-γ-treated cells the development of an antiviral effect, as well as the induction of IFN-induced enzyme activities and mRNAs, is blocked under conditions in which the synthesis of an intermediary protein is blocked. Kusari and Sen (15), in studies carried out to examine the regulation of the synthesis of the IFN-α-induced mRNAs, demonstrated that the induction of four IFN-induced mRNAs in HeLa cells required ongoing protein synthesis, whereas the induction of the IFN-induced mRNAs in other cells did not require ongoing protein synthesis. Thus, the regulation of the induction of these mRNAs is different among different IFN-α-treated cells. It is conceivable that the difference in the regulation of the IFN-induced mRNAs observed in different IFN-α-treated cells and the ability of some IFN-γ-treated cells to produce an IFN-induced protein and develop an antiviral effect, under conditions in which an intermediary protein cannot be produced, is due to the constitutive production in some cells of a regulatory protein which must be synthesized in other cells for them to respond to the IFNs.

Various investigators have reported the cloning of an mRNA which codes for an IFN-induced 56,000-dalton protein (6, 38). In an attempt to study the synthesis of the protein coded for by this mRNA, Cheng et al. (7) developed a polyclonal antibody to a 13-amino-acid peptide which would be coded for by this mRNA. These investigators report that the synthesis of this 56,000-dalton protein occurs in cells treated with either IFN-α or IFN-β, but not in cells treated with IFN-γ. The same observation has been made by others (15), who have examined the levels of this mRNA in IFN-treated cells. The fact that the protein we have studied is synthesized in IFN-α- and IFN-γ-treated cells strongly suggests that the IFN-induced protein to which we developed a monoclonal antibody is different from the IFN-induced 56,000-dalton protein studied by Cheng et al. (7) and that there exist two distinct IFN-induced 56,000-dalton proteins. In fact, high-resolution SDS-PAGE of S-10s prepared from IFN-α- and IFN-γ-treated cells reveals the presence of a protein, slightly larger than the 56,000-dalton protein we have studied, that is synthesized in IFN-α- but

not IFN-γ-treated cells. The function of these proteins and the relationship among them awaits further analysis.

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