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A MEMBRANE PROTEIN FROM IFN-β-TREATED DAUDI CELLS CAUSES A CESSATION IN CELL GROWTH

Milton C. Hillman, Jr., Ernest Knight, Jr., and Dale C. Blomstrom

Central Research & Development Department, Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, DE 19898

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SUMMARY: An interferon-induced 17 kDa protein has been partially purified from the membranes of interferon-β-treated Daudi cells. A fraction containing the 17 kDa protein purified 200 fold causes an inhibition of growth of Daudi, Namalva and Hela cells. The same fraction purified from the membranes of untreated cells causes no inhibition of cell growth. This interferon-β-induced protein is located on the exterior of Daudi cells for it can be labeled with [^{125}I] iodine catalyzed by lactoperoxidase. These results suggest that interferons induce a cell surface protein whose role is to cause an inhibition of cell growth.

The inhibition of cell growth by an interferon (IFN) was first described two-and-a-half decades ago (1), yet the molecular mechanisms involved in the IFN-induced growth inhibition remain obscure. This inhibition of growth is the reason the IFNs are being used clinically as antitumor agents. No genes or proteins have been directly implicated in the IFN-induced inhibition of cell growth. The induction of a membrane protein by IFN-β has been correlated with the IFN-induced cessation of cell growth (2); however, no evidence has been reported showing a direct role of this protein in growth regulation. We report here that an IFN-induced 17 kDa protein is located on the exterior surface of Daudi cells. Furthermore, we show that a fraction containing the 17 kDa protein, purified from the membranes of IFN-β-treated Daudi cells and devoid of IFN activity, will cause an inhibition of the growth of Daudi, Namalva and Hela cells. In contrast, the same fraction purified from the membranes of untreated Daudi cells caused no significant inhibition of cell growth. In our

Abbreviations:

IFN: interferon; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS: phosphate-buffered saline.

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original report, we estimated the molecular weight of the IFN-induced membrane protein to be 20 kDa. Recent analyses indicate that this protein is 17 kDa, and we refer to it hereafter as the 17 kDa protein.

MATERIALS AND METHODS

Cells: Daudi and Namalva cells were grown in suspension in RPMI 1640 medium supplemented with fetal calf serum. Hela cells were grown in monolayer in MEM supplemented with 7% horse serum.
Labeling of proteins with [^{35}S]-Methionine and [^{125}I]-Iodine: Daudi cell proteins were labeled with [^{35}S]-Methionine as previously described (2). Interferon-β-treatment was for 16 hr at 50 units/ml. Membrane fractions were prepared as described (2) and membrane pellets were dissolved in gel loading buffer and analyzed by SDS-PAGE on a 15% gel using the buffer system of Laemmli (3). Cell surface proteins of IFN-β-treated and untreated whole Daudi cells were labeled with [^{125}I]-Iodine catalyzed by lactoperoxidase. Briefly, 1 x 10^7 cells were labeled for 15 min at 25°C in 0.75 ml PBS containing 400 μg/ml lactoperoxidase, 120 μg/ml glucose oxidase, 0.02 M glucose and 400 μCi sodium iodide-[^{125}I]. Cells were washed 2X with cold PBS containing 0.01 M NaI prior to preparation of membranes. [^{35}S] and [^{125}I]-labeled proteins were analyzed by SDS-PAGE and visualized by autoradiography.
Purification of the 17 kDa protein: Purification of the IFN-β-induced 17 kDa membrane protein was by preparative electrophoresis from a fraction containing total membranes (2). The 17 kDa protein was recovered from the SDS-gel by electroelution (4). In a typical experiment, membrane proteins from 1 liter of IFN-β-treated or untreated Daudi cells were loaded onto 16 lanes of a slab gel. After electrophoresis, the 17 kDa region was cut out as a strip and the proteins were electroeluted. The electroeluted proteins were made 1% in fetal calf serum and dialyzed extensively versus RPMI 1640 growth medium containing 1% fetal calf serum. The dialyzed proteins were used in the growth inhibition experiments shown in Fig. 2 and Table 1. The eluted proteins had no detectable IFN antiviral activity (<1 unit/ml). Protein content of the electroeluted fractions was determined by the bicinchoninic acid method (5) prior to addition of fetal calf serum and dialysis.
Cell Growth Inhibition: The electroeluted 17 kDa fractions from membranes of untreated and IFN-β-treated Daudi and Namalva cells were used to determine their effect on the growth of Daudi, Namalva and Hela cells. Growth inhibition experiments were performed on exponentially growing Daudi and Namalva cells in 0.5 ml of RPMI 1640 containining 15% fetal calf serum. The 17 kDa protein fraction was added to cells at 30 x 10^4 cells/ml and cells were counted in a hemocytometer every 24 hr for three successive days. Hela cells were grown in 25 mm dishes and the 17 kDa protein fraction was added 16 hr after plating. After 48 hr and 72 hr cells from duplicate cultures were made monodisperse by trypsin treatment and counted.

RESULTS AND DISCUSSION

Prior to fractionation of the IFN-β-induced 17 kDa protein (Fig 1a), it was desirable to determine its cellular

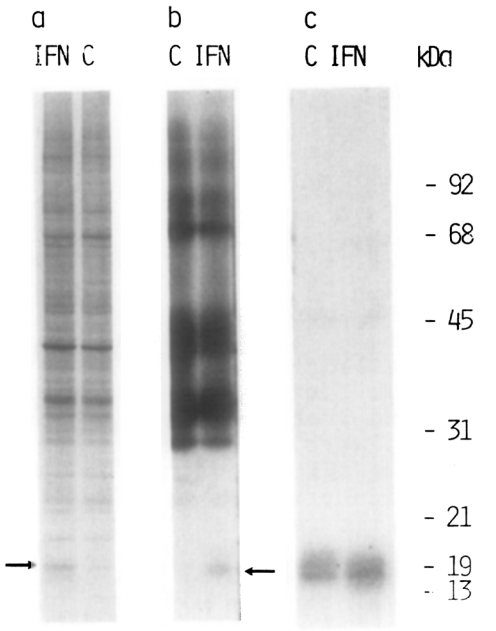

Fig. 1. SDS-PAGE analysis of [^{35}S]-Methionine and [^{125}I] Iodine-labeled membrane proteins. a. An autoradiogram of membrane proteins labeled with [^{35}S]-Methionine analyzed by SDS-PAGE. b. An autoradiogram of membrane proteins labeled with [^{125}I]-Iodine. c. Proteins eluted from the 17 kDa region of a preparative gel identical to the gel in a., analyzed on an analytical gel and stained with Coomassie Blue.

localization, e.g. plasma or interior membranes. This was accomplished by [^{125}I]-Iodine/lactoperoxidase catalyzed labeling of cell surface proteins. Analysis by SDS-PAGE of the [^{125}I]-labeled proteins in the membrane fraction revealed a 17 kDa protein from IFN-β-treated Daudi cells (Fig. 1b), but not from untreated cells, indicating that the IFN-induced 17 kDa protein is located, at least in part, on the exterior of the cell.

Preparative SDS-PAGE was used to purify the 17 kDa protein and to obtain sufficient quantities to determine if it would cause a cessation in cell growth when added to the culture medium of growing cells. Membrane proteins in the 17 kDa region of a SDS-gel were electroeluted as described in Materials and Methods. The brackets in the autoradiogram of Fig. 1a show the region that was cut out and electroeluted from an identical preparative gel. The proteins in this region were electroeluted from gels containing membrane proteins from IFN-β-treated and untreated cells, respectively. The electroeluted proteins from each gel are shown by Coomassie Blue staining of an analytical gel in Fig. 1c. We have designated the proteins shown in Fig. 1c the 17 kDa

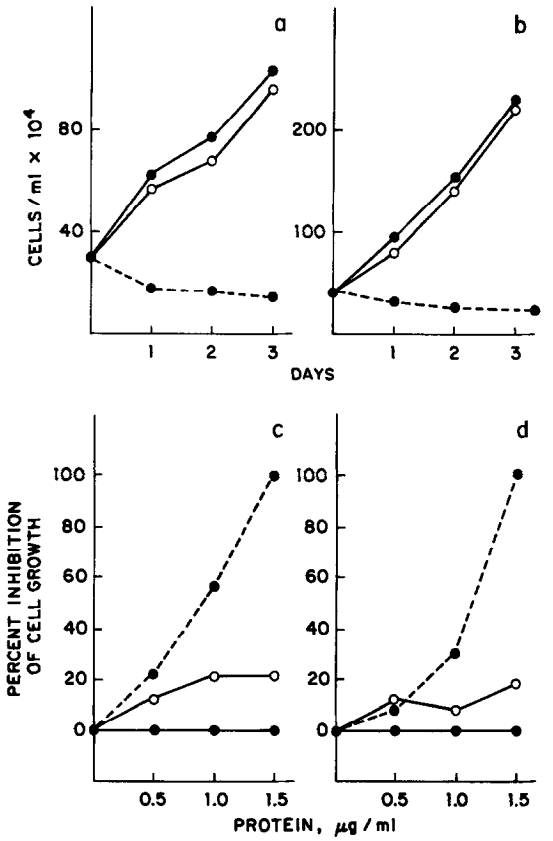

Fig. 2. Inhibition of growth of Daudi and Namalva cells by the electroeluted 17 kDa protein fraction. a. Time course of the effect of the 17 kDa protein fraction on the growth of Daudi cells; 1 µg/ml. b. Time course of the effect of the 17 kDa protein fraction on the growth of Namalva cells; 1 µg/ml. c. Effect of concentration of 17 kDa protein fraction on the inhibition of Daudi cell growth. d. Effect of concentration of 17 kDa protein fraction on the inhibition of Namalva cell growth. —●—●— no additions, —○—○— protein from untreated cells, --●--●-- protein from IFN-β-treated cells.

protein fraction. In all growth inhibition experiments, equal amounts of electroeluted proteins (from untreated and IFN-β-treated cells) were added. The 17 kDa protein fraction from IFN-β-treated Daudi cells caused a complete inhibition of the growth of both Daudi (Fig. 2a) and Namalva (Fig. 2b) cells. In contrast, the eluted proteins from the membranes of untreated cells caused no significant inhibition of growth (Fig. 2a & 2b). We then determined that the inhibition of cell growth by electroeluted proteins was concentration-dependent for both Daudi and Namalva cells (Fig. 2c & 2d). Complete inhibition in these experiments was observed at 1.5 µg/ml of added protein. Again, only minimal growth inhibition was observed by the proteins

purified from the membranes of untreated Daudi cells (Fig. 2c & 2d). These data show that Namalva cells can respond to the 17 kDa protein fraction prepared from Daudi cells, and therefore suggest that the reason that the growth of Namalva cells cannot be inhibited by IFN is that they cannot synthesize the 17 kDa protein (6).

A summary of the experiments on growth inhibition by membrane proteins from IFN-β-treated cells is shown in Table 1. The electroeluted 17 kDa protein fraction from the membranes of IFN-β -treated Daudi cells also caused a growth inhibition of Hela cells (Table 1). We also show in Table 1 that the 17 kDa protein fraction from either untreated or IFN-β-treated Namalva cells - a cell insensitive to the growth inhibitory effects of IFN (6) and unable to synthesize the 17 kDa protein in response to IFN - causes no significant growth inhibition of either Daudi or Namalva cells.

We believe the growth inhibitory activity resides in a protein since: (a) it migrates like a protein on SDS-PAGE, (b) it does not pass through a dialysis membrane, and (c) the activity is substantially reduced by proteolytic enzyme treatment (Table 1). The growth inhibitory activity is not IFN-β since the 17 kDa fraction contains no antiviral activity and the 17 kDa fraction inhibits the growth of IFN-insensitive Namalva cells. Furthermore, IFN-β migrates much slower than the 17 kDa protein on SDS-PAGE and would not appear in the 17 kDa electroeluted fraction.

We conclude that the 17 kDa protein induced by IFN-β or -α is located on the exterior of Daudi cells. This protein has been purified approximately 200 fold over the original membrane fraction by SDS-PAGE. A fraction containing it, electroeluted from a SDS-gel, inhibits the growth of Daudi, Namalva, and Hela cells. A similar 17 kDa fraction purified from the membranes of untreated Daudi cells caused no significant inhibition of growth of these three cell lines (Fig. 2 and Table 1). We do not know at present if the growth inhibitory activity is due solely to the IFN-induced 17 kDa protein or if additional undetected IFN-induced membrane proteins in the same fraction are necessary. Purification of the 17 kDa protein to homogeneity is essential to determine if it is solely responsible for growth inhibition. The mechanism of the growth inhibition is not evident from the experiments reported here. We speculate that the purified 17 kDa

Table 1

Inhibition of growth of Daudi, Namalva and Hela cells by electroeluted membrane proteins from IFN-β-treated Daudi cells

Membrane Preparation	Source of Membranes	IFN Treatment[a]	Target Cell	Cells/ml[b] x 10^4	Percent Inhibition of Growth
1	-[c]	-	Daudi	110	
1	Daudi	-	Daudi	100	14
1	Daudi	+	Daudi	40	100
2	-	-	Namalva	200	
2	Daudi	-	Namalva	145	33
2	Daudi	+	Namalva	72	76
3	-	-	Daudi	101	
3	Daudi	-	Daudi	98	5
3	Daudi	+	Daudi	15	100
3	-	-	Namalva	210	
3	Daudi	-	Namalva	200	5
3	Daudi	+	Namalva	20	100
3[d]	Daudi	+	Namalva	160	28
3	-	-	HeLa	250	
3	Daudi	-	HeLa	264	0
3	Daudi	+	HeLa	136	65
4	-	-	Namalva	126	
4	Daudi	-	Namalva	129	0
4	Daudi	+	Namalva	31	100
5	-	-	Namalva	218	
5	Daudi	-	Namalva	191	15
5	Daudi	+	Namalva	89	72
6	-	-	Daudi	119	
6	Namalva	-	Daudi	95	30
6	Namalva	+	Daudi	93	33
6	-	-	Namalva	251	
6	Namalva	-	Namalva	234	8
6	Namalva	+	Namalva	240	5

Growth inhibition studies were performed on target cells as described in Fig. 2a. [a]A plus(+) in this column denotes treatment of cells with IFN-β for 16 hr. prior to preparation of membranes. [b]Cell count 72 hr after addition of membrane proteins. Membrane proteins were added to cells at 30 x 10^4 cells/ml. Electroeluted membrane proteins from untreated and IFN-β-treated cells were added to a concentration of 1 μ_dg/ml. [c]a minus(-) in this column denotes no addition to cells. [d]The 17 kDa fraction from membrane of IFN-treated cells was treated for 20 hr at 37° with V-8 protease at a 10:1 ratio of eluted protein to V-8 protease. V-8 protease has no effect on cell growth at the concentrations used.

protein inserts itself into the plasma membrane in the same location as it occupied originally, thereby causing a cessation of cell growth by an unknown mechanism.

Recent reports have suggested that one mechanism by which IFNs may inhibit cell growth is by the reduction in cell surface binding of growth factors. Treatment of K-562 and Daudi cells with IFN-α reduced the number of transferrin binding sites per cell but did not affect receptor affinity (7). IFN-α2 treatment of MDBK cells caused a decrease in the number of receptors for epidermal growth factor (EGF) and caused a reduction in affinity of EGF for its receptor (8). The down-regulation of insulin receptors on Daudi cells has been shown to occur after treatment of the cells with IFN-α (9). All three groups suggested that an IFN-induced reduction in growth factor binding may be a mechanism by which the IFNs cause a cessation in cell growth. It is possible that the 17 kDa protein causes, directly or indirectly, a reduction in binding of essential growth factors, thereby causing a cessation of growth. The 17 kDa protein is not an IFN-enhanced class I or class II HLA antigen since the HLA antigens have molecular weights substantially larger, 28,000-48,000, than the 17 kDa protein (see ref. 10 for review). What is clear from our experiments is that a membrane protein (or proteins) of approximately 17 kDa, purified from IFN-β-treated Daudi cells causes an inhibition of cell growth when added to the culture medium of IFN-growth-sensitive and IFN-growth-insensitive cells. Purification and characterization of the 17 kDa protein should aid in understanding the molecular mechanisms involved in the IFN-induced cessation of cell growth.

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