Expression Cloning of an Interferon-inducible 17-kDa Membrane Protein Implicated in the Control of Cell Growth*

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Interferon-inducible membrane proteins of approximately 17 kDa have been suggested to play a role in the antiproliferative activity of interferons based on (1) their pattern of induction in interferon-sensitive and -resistant cell lines and (2) the ability of a membrane fraction enriched in 17-kDa proteins to inhibit cell growth. To gain insight into the nature of the proteins that mediate the antiproliferative activity of interferons, a monoclonal antibody, 13A5, was generated that reacted specifically with a 17-kDa interferon-inducible cell surface protein. The expression pattern of this 17-kDa protein by human cell lines correlated with sensitivity to the antiproliferative activity of interferons. To obtain information regarding the structure of this protein, the 13A5 antibody was used to screen COS cells transfected with a human cDNA expression library. Sequence analysis of a full-length cDNA clone revealed identity with the 9–27 cDNA, previously isolated on the basis of its interferon inducibility by differential screening. In addition, the 17-kDa protein encoded by the 9–27 gene was shown to be identical to the Leu-13 antigen. Leu-13 was previously identified as a 16-kDa interferon-inducible protein in leukocytes and endothelial cells and is a component of a multimeric complex involved in the transduction of antiproliferative and homotypic adhesion signals. These results suggest a novel level of cellular regulation by interferons involving a membrane protein, encoded by the interferon-inducible 9–27 gene, which associates with other proteins at the cell surface, forming a complex relaying growth inhibitory and aggregation signals.

Interferons (IFN)1 are multifunctional cytokines that play a critical role in the defense against viral or parasitic infections. These cytokines also exhibit antiproliferative and differentiat-

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‡The abbreviations used are: IFN, interferon; mAb, monoclonal antibody; FCS, fetal calf serum; PBS, phosphate-buffered saline; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonic acid; PBMC, peripheral blood mononuclear cells.
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...molecule-like cells (histiocytic leukemia). These cell lines were grown in
FS-7F (human fibroblastoid cells) were grown at 37°C in a humidified
Synthesis of 1–8u cDNA—The 1–8u cDNA was obtained by reverse
Expression of membrane antigens was measured by indirect immunofluorescence analysis on a FACSscan flow cytometer (Becton Dickinson). Briefly, 106 cells were washed 3 times with PBS and incubated in PBS, 0.5% bovine serum albumin, 0.01% NaN3, 1mM phenylmethylsulfonyl fluoride, 100 units/ml aprotinin, 10
Cell surface protein expression was measured by flow cytometry
Fluorocytometric Analysis—Expression of membrane antigens was measured by indirect immunofluorescence analysis on a FACSscan flow cytometer (Becton Dickinson). Briefly, 106 cells were washed 3 times with PBS and incubated in PBS, 0.5% bovine serum albumin, 0.01% NaN3, 1mM phenylmethylsulfonyl fluoride, 100 units/ml aprotinin, 10

RESULTS

Generation of a Monoclonal Antibody Reactive with a 17-kDa Membrane Protein Induced by IFN-α on Daudi Cells—Daudi cells treated with IFN-α were used as a source of IFN-induced antigens since they are very sensitive to the antiproliferative activity of type I IFNs (13). To increase the frequency of mAbs recognizing IFN-α-induced antigens, mice were first injected with untreated Daudi cells and submitted to a cyclophosphamide regimen in an attempt to selectively kill lymphocytes stimulated by constitutively expressed Daudi antigens. Tolerization was followed by immunization with IFN-α-treated Daudi cells. Splenocytes were fused to Sp2/0 myeloma cells, and the resulting hybridomas were screened by indirect immunofluorescence for secretion of mAbs reacting differently with IFN-α-treated and untreated Daudi cells.

One mAb termed 13A5, an IgMκ, reacted with a protein that was highly inducible by IFN-α on Daudi cells. The antigen recognized by this mAb was characterized by immunoprecipitation of 125I-labeled surface proteins from IFN-α-treated or
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Fig. 1. Immunoprecipitation of Daudi cells' proteins by 13A5. Un-
treated (−) or IFN-α-treated (+) Daudi cells were 125I-labeled and lysed in Non-
idet P-40 buffer. Immunoprecipitates or crude lysates were resolved on 20% poly-
acrylamide gel. A, immunoprecipitation of cell lysates with 13A5 or a control mAb. B, longer exposure of the gel shown in A. C, crude lysates. Molecular mass markers are indicated in kDa on the left.

Expression of the 17-kDa Protein in Response to Interferons—Established human cell lines differ widely in their sensitivity to the antiproliferative activity of IFNs. Expression of the 17-kDa protein in response to type I and II IFNs was assayed by indirect immunofluorescence on a panel of IFN-sensitive and -resistant cell lines. Representatives of epithelial, fibroblastic, promyelomonocytic and lymphoblastoid (B, T, and null) origin were included (Table I). DIF8 and Daudi R are mutant cell lines derived from Daudi cells that were selected for resistance to the antiproliferative effect of IFN-α (13, 14). U937, Raji and Namalwa cell growth is also insensitive to the inhibitory effects of IFN-α.

The results of the 17-kDa protein expression analysis are presented in Table I. Fig. 2 shows the fluorescence histograms of four representative cell lines on a logarithmic scale. We observed that IFN-α treatment increased the expression of the 13A5 antigen on most of the cell lines tested. As much as a 20-fold increase was observed on Daudi, J urkat, Reh, and FS-7F cells. Interestingly, the level of induction was very weak on several resistant cell lines (e.g. on DaudiR or U937) or undetectable on DIF8. The pattern of expression appeared to correlate closely with the ability of IFN-α to inhibit cell growth. IFN-γ also significantly affected the expression of the 17-kDa protein although to a lesser extent than did IFN-α. Induction of the 17-kDa protein by IFN-γ was observed in most cell lines tested, except in those derived from B or T cells. This result is consistent with our previous observation that genes that are predominantly induced by type I IFN are not or very poorly inducible by IFN-γ in cell lines of lymphoblastoid origin (26). We concluded that the 17-kDa protein is inducible by both types of IFNs on sensitive cells.

Some of the cell lines tested expressed a basal level of the 17-kDa protein, which was particularly prominent on K562 cells. Confirming the fluorocytometric analysis, immunoprecipitation of 125I-labeled surface proteins with 13A5 showed the highest basal level of the 17-kDa protein on K562 cells (Fig. 3). Overexposure of the autoradiogram allowed the detection of a 28-kDa component, similar to the one observed in the proteins immunoprecipitated by 13A5 from IFN-α-treated Daudi cells (compare Figs. 1B and 3). Immunoprecipitated proteins of 17 and 28 kDa were also detected under nonreducing conditions (Fig. 3B). These data suggest that the 17-kDa protein is non-covalently associated in the cell membrane with a protein of 28 kDa.

Expression Cloning of the cDNA Coding for the 17-kDa Protein—we constructed a directional cDNA expression library in the psc1 vector (21) using mRNA extracted from HeLa cells that had been incubated for 24 h in the presence of IFN-α. Screening of transfected cells reactive with the 13A5 mAb was undertaken using the “panning” method previously described for the cloning of various eukaryotic membrane proteins (17, 23, 24, 27). Six successive amplification and enrichment cycles.
were performed. Each consisted of 4 steps: (1) transfection of the library into COS cells, (2) selection of the positive cells by panning, (3) isolation of the plasmid DNA by Hirt extraction, (4) amplification of the recovered plasmid DNA in bacteria. Three additional screening cycles were performed by transf ecting plasmid extracted from counted colony pools and by detecting the positive pools by indirect immunofluorescence. This procedure lead to the isolation of two identical clones containing a cDNA insert of 650 base pairs. COS cells transf ected with the cDNA from one of these clones was strongly reactive with the 13A5 mAb, as determined by flow cytometric analysis (Fig. 4A).

The 9–27 Gene Encodes Leu-13—Leu-13 is a 16-kDa leukocyte surface antigen whose expression is stimulated on endothelial cells and B lymphocytes after exposure to both types of IFNs (4, 5, 10, 30). Previous immunoprecipitation studies have established that Leu-13 is noncovalently associated with polypeptides of 28, 48, and 90 kDa (10, 12). Taken together, these observations prompted us to determine if the 9–27 gene, or the closely related 1–8u or 1–8d genes, encoded the Leu-13 antigen. In the experiment shown in Fig. 4A, COS cells transf ected with the 9–27 cDNA were first reacted with 13A5 and anti-Leu-13 mAbs and then were counterstained with a fluorescein-conjugated antibody and analyzed by flow cytometry. B, same experiment as in A except that cells were transf ected with the pse1.1–8 plasmid (heavy lines). The control plasmid (thin lines) was pse1.

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lymerase chain reaction approach using primers designed to amplify the 1–8u and 1–8d mRNAs resulted in the obtention of the 1–8u but not of the 1–8d cDNA, which is less abundant and less homologous. As shown in Fig. 4A, neither anti-Leu-13 nor 13A5 reacted with COS cells transfected with the 1–8u cDNA. These results were confirmed by immunoprecipitation experiments performed on in vitro synthesized 9–27 and 1–8u proteins. Both mAbs were able to precipitate 9–27 but not 1–8u products (data not shown). Therefore, the epitope recognized by 13A5 and anti-Leu-13 on the 9–27 protein is not present in the 1–8u protein. Thus, we conclude that the leukocyte antigen, Leu-13, is composed of only one polypeptide encoded by the IFN-inducible 9–27 gene.

Proteins Coprecipitated with the 17-kDa Protein—We next investigated whether the 28-kDa protein coprecipitated with the 17-kDa protein by 13A5 could correspond to TAPA-1. TAPA-1 is a 26-kDa protein that can associate with Leu-13 (8, 9, 11, 12). 125I-labeled K562 cells were lysed in a 2% Nonidet P-40 buffer, as described previously in Fig. 3, or in buffer containing 1% CHAPS, a mild nonionic detergent, to preserve most of the noncovalent protein interactions. Fig. 5A shows that the protein whose interaction with 17-kDa protein is preserved in the Nonidet P-40 lysate did not correspond to TAPA-1. However, when the cells were lysed in the milder CHAPS buffer, several proteins were precipitated with either 5A6, the anti-TAPA-1 mAb, or 13A5, the anti-9–27-encoded 17-kDa protein mAb. Using the 5A6 mAb, TAPA-1 was detected by Western blot among the proteins precipitated with the 13A5 mAb in CHAPS lysate (Fig. 5B), in agreement with previously reported results obtained using anti-Leu-13 mAb (8, 9, 11, 12). The 28-kDa protein coprecipitated by 13A5, but not by 5A6, from Nonidet P-40 lysate appeared in both precipitation products when CHAPS lysate was used. Thus, 9–27/Leu-13 is associated with several proteins at the cell surface, including TAPA-1, and the strength of these interactions can be probed using different detergents.

Effect of 13A5 and anti-Leu-13 mAbs on Homotypic Adhesion and Proliferation—Previous studies using anti-Leu-13 mAb have established that this mAb can induce the homotypic adhesion of cells expressing Leu-13 (4, 5, 10) and can inhibit the anti-CD3-driven proliferation of PBMC (4, 12, 17). Fig. 6A shows the results of an homotypic adhesion assay on U937, Jurkat, and K562 cells using 13A5, anti-Leu-13, and control mAbs. Interestingly, the 13A5 mAb did not induce cell adhesion. In contrast, the anti-Leu-13 mAb triggered adhesion of cells expressing 9–27/Leu-13, either constitutively (K562 or Jurkat), or in response to IFN treatment (U937). Similarly, the 13A5 mAb had no significant effect on the anti-CD3-driven proliferation of human PBMC. Human PBMC were cultured in the absence or presence of 0.5 μg/ml of anti-CD3 mAb. Anti-Leu-13, 13A5, or isotype-matched control antibodies were included in cultures at a concentration of 10 μg/ml. [3H]Thymidine incorporation was measured on day 3 of culture. Results represent the mean ± S.D. of replicate cultures. Data are representative of three independent experiments.
biological processes. Several proteins whose synthesis is induced by IFNs have been implicated in the antiviral activity shared by all IFNs (for review, see Refs. 2 and 31–33). The inhibition of cell growth by IFNs is also likely to result from an action on multiple pathways affecting different steps at which cell growth can be regulated. Interestingly, two double-stranded RNA-dependent antiviral pathways induced by IFNs have been recently implicated in their antiproliferative activity as well (34–36). IFN treatment also directly affects the function of two genes known to be involved in the control of the cell cycle, the proto-oncogene c-myc and the tumor-suppressor retinoblastoma gene, resulting in arrest at the G1/S phase of the cell cycle (37–40).

It has been shown that the antiproliferative activity of IFN could be transferred from cell to cell, not by diffusion of a soluble mediator but through direct contact between cells (3). To explore the role of membrane proteins in the antiproliferative activity of IFNs, we generated mAbs directed against IFN-inducible membrane antigens. We obtained an antibody that reacted specifically with a 17-kDa protein. This 17-kDa protein was induced by type I and II IFNs on a wide range of cell lines sensitive to the antiproliferative effect of IFNs, but not significantly on resistant cell lines. Screening of an expression library yielded a full-length cDNA clone encoding the 17-kDa protein, and sequence analysis revealed identity with the 9–27 cDNA.

The 9–27 gene is induced by both type I and II IFNs, and the mRNA level can increase as much as a 100-fold upon induction by IFNs. Induction by IFNs is transcriptionally controlled by a single IFN-stimulated response element present in the promoter of the gene (41). The protein encoded by the 9–27 gene has a calculated molecular mass of 13.9 kDa, in close agreement with the observed 16–17 kDa (7, 10). In addition, we demonstrated that 9–27 was coding for the leukocyte antigen Leu-13. In most cell types studied thus far, Leu-13 was shown to be part of a membrane complex containing several distinct proteins. One of these proteins is the 26-kDa TAPA-1 protein, the target of an antiproliferative antibody (12). TAPA-1 is strongly related to the sequence level to two other surface proteins involved in the regulation of cell growth, the ME491 melanoma-associated antigen, and CD37, another leukocyte antigen (17). Leu-13 and TAPA-1 are involved in a mechanism controlling cellular adhesion since anti-Leu-13 and anti-TAPA-1 mAbs each triggers a general homotypic adhesion phenomenon. Interestingly, homotypic adhesion induced by anti-Leu-13 or anti-TAPA-1 is not dependent on adhesion pathways involving known adhesion molecule including the leukocyte function-associated antigen-1, the intercellular adhesion molecule-1, CD44, or VLA-4 (4, 5, 12).

Along with their involvement in the regulation of cell aggregation, Leu-13 and TAPA-1 are likely to also play a role in the control of cell growth. Indeed, anti-Leu-13 and anti-TAPA-1 mAbs can directly inhibit cell growth, although this effect is observed in a more restricted subset of cell lines (4, 12, 17). Furthermore, anti-Leu-13 mAbs were shown to potentiate the antiproliferative effect of IFN-α on leukemic B cells (4). Insensitivity to the growth inhibitory effect of anti-Leu-13 and anti-TAPA-1 mAbs in some cell lines can probably be accounted for by the alterations in the control of cell growth that occur when a cell line is established for in vitro growth. We also attempted to establish cell lines stably expressing 9–27/Leu-13 under the control of a constitutive promoter. Although some clones did express heterogeneous levels of the protein early in the selection procedure as revealed by staining, expression rapidly declined to undetectable levels (data not shown). This result suggested that stable expression of 9–27/Leu13 might be hindering cell growth.

Evidence that antibodies against 9–27/Leu-13 and TAPA-1 can induce a biological response suggests that the multimeric cell surface complex containing 9–27/Leu-13, TAPA-1, and other molecules is a receptor for an as yet unidentified ligand. Indeed, activation of a signal transduction pathway by extracellular signals often requires ligand-induced dimerization or oligomerization of the corresponding receptor. Kinase(s) associated with the receptor are brought together, resulting in their reciprocal phosphorylation and the subsequent activation of further downstream components of the signal transduction pathway (42). Therefore, antibodies against cell surface receptors that are activated by dimerization can sometimes function as an agonist because they artificially induce dimerization of the receptor, whereas Fab fragments of such antibodies have no activity (42).

Taken together, these results show that the 9–27 gene is coding for a cell surface protein that associates with other membrane proteins, forming a multimeric complex that relays growth inhibitory and cell adhesion signals.

Interferons seem to exert their inhibition on cell growth by acting at many different levels, (1) directly affecting the function of protein such as c-myc and Rb that are intimately involved in cell-cycle control, (2) increasing the level of enzymes such as the double-stranded RNA-dependent protein kinase and the 2–5A synthetases that inhibit cell anabolism, and (3) inducing cell surface proteins such as 9–27/Leu-13 that relay other growth inhibitory signals. While the activation of a single pathway might be sufficient to inhibit the growth of a given cell, activation of multiple pathways by IFNs allows for both more efficiency and flexibility in the control of cell growth. Indeed, in a physiological setting, IFNs are acting on a population of cells at distinct stages and in different programs of differentiation, cells that are continuously exposed to various other stimuli and have to maintain the ability to perform other essential functions, hence the requirement for efficiency and flexibility, i.e. multiple pathways.

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