Effects of Interferon-γ on Expression of Cell Surface Receptors for Collagen and Deposition of Newly Synthesized Collagen by Cultured Human Lung Fibroblasts

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Abstract

We used cultured human diploid lung fibroblasts as a model system to examine the effects of recombinant IFN-γ on synthesis of collagen, matrix deposition of newly synthesized collagen, and the expression of cell surface receptors for collagen. Using [^{3}H]proline-labeled cells we found that IFN-γ resulted in dose-dependent inhibition of fibroblast collagen synthesis. Pulse-chase experiments to analyze compartmentalization of newly synthesized collagen showed that the decrease in collagen synthesis was confined to the soluble pool of procollagen in the medium, while extracellular matrix associated collagen was not changed, indicating that a larger proportion of newly synthesized collagen was deposited into the matrix in IFN-γ exposed fibroblasts (34.2 vs. 25.3%). This increase in the efficiency of collagen matrix deposition was associated with enhanced expression of a cell surface receptor for collagen as detected by indirect immunofluorescence labeling and analysis by flow cytometry. Fibroblasts (IMR-90) cultured in the presence of IFN-γ (1,000 U/ml) exhibited a twofold increase in mean linear fluorescence intensity compared with cells cultured under control conditions. The distribution of log fluorescence intensity in both control and IFN-γ exposed cells was normally distributed about the mean, indicating that discrete subpopulations with respect to receptor expression were not present. Increased fluorescence intensity and log normal distribution of fluorescence intensity also were identified in IFN-γ-treated lung fibroblasts from a normal adult individual and two strains obtained from patients with pulmonary fibrosis. These results indicate that IFN-γ modulates fibroblast collagen matrix deposition as well as collagen synthesis. The associated increase in collagen receptors suggests that cytokine-mediated modulation of the cell surface may be a contributing factor in regulation of fibroblast collagen accumulation in the extracellular matrix or in cellular interaction with collagen-containing matrix. Such an effect could modulate the interaction of fibroblasts with extracellular matrix at sites of inflammation and play an important role in the remodeling of matrix during repair from tissue injury.

Introduction

Resident lung fibroblasts are thought to be responsible for the normal production and maintenance of the connective tissue matrix and for repair after lung injury (1, 2). Fibroblasts also assume a central role in diseases, such as diffuse idiopathic pulmonary fibrosis and pulmonary sarcoidosis, that are characterized by excessive collagen deposition in the lung interstitium (2, 3). Because of the prominance of inflammatory cells associated with lung connective tissue remodeling in these conditions, the role of lymphokines and monokines in the regulation of fibroblast collagen synthesis and deposition has been a subject of recent interest (4, 5).

Regulation of procollagen synthesis at transcriptional and translational levels has been recognized as a means of modulating collagen accumulation in the extracellular matrix (1, 6, 7). The biosynthesis of collagen also involves a number of cotranslational and posttranslational modifications, both intracellular and extracellular, that influence the secretion and assembly of the procollagen molecules (8). The deposition of newly synthesized collagen into stable matrix is also potentially regulated by interactions of the cell surface and by interactions with other macromolecules in the extracellular matrix (9–13). A family of cell surface receptors, the integrins, that recognize a variety of extracellular matrix macromolecules has recently been identified (14–19). These receptors appear to play a pivotal role in cell adhesion to the extracellular matrix, and a role in matrix assembly has also been suggested (20–22).

We initiated these experiments to examine the role of IFN-γ in modulation of lung fibroblast collagen matrix deposition. Our interest was directed to IFN-γ, a product of activated T lymphocytes, because this cytokine has been shown to mediate changes in collagen synthesis (23–25) as well as mesenchymal cell surface proteins (26–32). We found that, although fibroblast total collagen synthesis is decreased by IFN-γ, a larger proportion of newly synthesized collagen is deposited into extracellular matrix. We also report that IFN-γ acts on fibroblasts to increase surface expression of a collagen receptor. These data suggest that IFN-γ may act on lung fibroblasts to modulate extracellular collagen deposition as well as collagen synthesis. The increase in collagen receptors suggests that modulation of the cell surface by IFN-γ may be a contributing factor in regulation of fibroblast collagen accumulation in the extracellular matrix as well as in cell adhesion to collagenous matrix.

Methods

Cell cultures. Human fetal lung fibroblasts (IMR-90) were obtained from the Human Aging Cell Repository (Camden, NJ) and used in the 9th–12th passage. Human adult lung fibroblasts were obtained from explant culture of tissue obtained at lung biopsy (diffuse idiopathic pulmonary fibrosis strains, M.W. and G.N., passage 6) or thoracotomy (normal adult lung fibroblast, 2599N, passage 11, kindly provided by Dr. Ganesh Raghu, University of Washington, Seattle, WA). Cultures were free of mycoplasma as detected with 4,6-diamidino-2-phenylindole staining. Cells were cultured in DME supplemented with 10% FCS, antibiotics (200 U/ml penicillin and 200 μg/ml streptomycin),

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Received for publication 17 June 1988 and in revised form 9 November 1988.

J. Clin. Invest.

0021-9738/89/05/1505/07 $2.00
Volume 83, May 1989, 1505–1511

and 2 mM glutamine. Confluent 60-mm plates of cells were then incubated with 0.4% DME-containing serum, 25 μg/ml ascorbic acid that was supplemented daily, and 0–1,000 antiviral units/ml human recombinant IFN-γ (a gift from Hoffman-LaRoche, Nutley, NJ).

Protein labeling. After exposure to IFN-γ, the cells were incubated with fresh serum-free DME containing 25 μg/ml ascorbic acid and [^{3}H]proline (20 μCi per plate) for 18 h. The labeling medium was removed and analyzed as described below. The cells were incubated for an additional 4 h in isotope-free chase medium to eliminate intracellular [^{3}H]proline from fibroblast cell layers. The chase medium was removed, the cell layers were rinsed with PBS and scraped from the plates.

Cell layer and medium samples, analyzed separately, were added to tubes containing protease inhibitors to yield, in final concentration, 1 mM *N*-ethylmaleimide, 2 mM EDTA, and 3 mM PMSF, and dialyzed for 18 h against running tap water. The samples were then hydrolyzed in 6 N HCl at 110°C for 18 h and dried in a centrifugal evaporator. The [^{3}H]hydroxyproline and [^{3}H]proline in the samples were separated chromatographically as previously described (33, 34), and total radioactivity in the corresponding peaks was determined by scintillation counting. To determine the specific radioactivity of hydroxyproline in newly synthesized procollagen, a portion of the hydrolyzed medium samples was derivatized with phenylisothiocyanate and analyzed by reversed-phase HPLC (model 5000; Varian Instruments) (35). The position and total content of hydroxyproline was determined by monitoring at 254 nm and comparison with hydroxyproline standards. Radioactivity was determined in aliquots of the corresponding peak.

Radiolabeled proteins were also examined by SDS-PAGE to assess the relative degree of enzymatic processing of procollagen to collagen and intermediate forms. After incubation with IFN-γ and radiolabeling as described above, the medium was removed, proteases were added, and ^{3}H-labeled procollagens were precipitated with 176 mg/ml of ammonium sulfate at 4°C. The precipitate was recovered by centrifugation at 20,000 *g* for 60 min, and examined after reduction with 10 mM DTT by SDS-PAGE using 6–10% gradient gels (36). The radiolabeled proteins were visualized by fluorography, and their migration was compared with that of purified human procollagen α_1 (I) and procollagen α_2 (I) chains as well as pepsin digested type I procollagen (36, 37). The relative concentrations of newly synthesized procollagen and processed forms were determined by scanning densitometry.

Antibodies. A murine MAb to a collagen-specific cell surface receptor designated class II, was prepared as previously described (18). Briefly, mice were immunized with nontrypsinized human HT-1080 fibrosarcoma cells. Immune spleen cells were fused with myeloma cells, and the resulting heterokaryons were screened for (*a*) reaction with HT-1080 cells in a solid phase assay, (*b*) immune precipitation of iodinated cell surface proteins of HT-1080 cells, (*c*) inhibition of cell adhesion to fibronectin- or collagen-coated styrene plates. The antibody used in these studies (P1H5) precipitated a single cell protein (under nonreducing conditions) with two subunits, α and β, with molecular masses of 145 and 125 kD, respectively. The antibody inhibited cell adhesion to types I, III, IV, V, and VI collagens, but not to fibronectin.

A murine MAb (MAb 60.5) directed against a framework class I MHC antigen, and a murine MAb (1F5) directed against specific B cell surface antigen (B1) were gifts from Dr. John Hansen of the Fred Hutchinson Cancer Research Center, Seattle, WA. A murine MAb to the platelet-derived growth factor receptor was provided by Dr. Daniel Bowen-Pope of the University of Washington, Seattle, WA.

Immunofluorescence detection of cell surface antigens. The expression of cell surface antigens was studied by indirect immunofluorescence labeling and flow cytometric analysis on a FACS. Confluent fibroblast monolayers were detached by incubation for 10 min at 37°C in 1 ml of 0.05% buffered trypsin. In one experiment, cells were detached by incubation for 10 min at 37°C in 3 mM EDTA in PBS.

Cells were washed in DME containing 10% FCS. Approximately 5 × 10^5 cells were incubated with 100 μl of MAb at 4°C for 60 min. For antibody P1H5, undiluted hybridoma supernatant fluid was used; for antibodies 60.5 and B-1, ascites fluid (1:250 dilution) was used. After washing twice in DME containing 10% serum, 150 μl of fluorescein isothiocyanate–conjugated goat anti-mouse antibody (diluted 1:60) (Tago, Inc., Burlingame, CA) was added and the incubation continued for an additional 30 min. Cells were then washed twice with PBS and suspended in 0.5 ml PBS for immediate analysis. Nonspecific binding was assessed in cells labeled with the fluorescein isothiosyanate–conjugated antibody and the irrelevant B cell antibody or no antibody.

Flow cytometry was performed using a FACS-II (Becton Dickinson Immunocytometry Systems, Mountain View, CA) flow cytometer equipped with a Cn argon laser (model 2W; Spectra-Physics, Mountain View, CA). Cells were excited with 300 mW of 488-nm light, and forward scatter and side scatter laser light were collected on a linear scale. After exclusion of debris (small, forward scatter events), data were collected in list mode, and reprocessed using the Consort 40 software package provided with the flow cytometer. Mean values of log fluorescence distributions were computed. Specific fluorescence intensity was expressed as the difference between the mean channel number of cells incubated with and without fluoresceinated IgG.

Results

Newly synthesized collagen was analyzed in human diploid lung fibroblasts (IMR-90) after labeling with [^{3}H]proline. IFN-γ exposure resulted in decreased collagen synthesis but not in total protein synthesis (Fig. 1). The concentration of IFN-γ used in these experiments was shown in preliminary dose-response studies to cause maximum decreases in [^{3}H]-hydroxyproline production. Decreased [^{3}H]hydroxyproline production by IFN-γ exposed fibroblasts was detected as early as 24 h after IFN-γ exposure and was maximal after 72 h of continuous exposure. A detailed analysis of the effect of IFN-γ on total protein and collagen production after 72 h of exposure is shown in Table I. The incorporation of [^{3}H]proline and production of [^{3}H]hydroxyproline were inhibited to 89 and 65% of control values, respectively, by IFN-γ. Thus, while radioactivity in both total protein and collagen synthesis were inhibited, collagen was suppressed to a greater extent, indicating a selective effect of IFN-γ on collagen synthesis.

To determine whether the decrease in [^{3}H]-labeled proteins

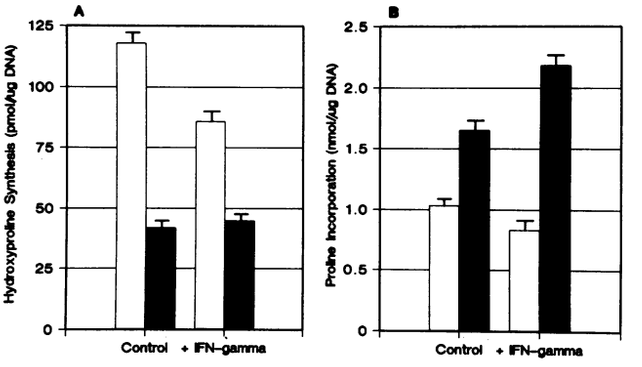

Figure 1. Effect of IFN-γ on collagen and total protein synthesis by human diploid lung fibroblasts. IMR-90 fibroblasts were incubated under control conditions or with 1,000 U/ml IFN-γ for 72 h and then labeled with [^{3}H]proline for 18 h. After a 4-h chase period in isotope-free medium, the cell layers (*solid bars*) and labeling medium (*open bars*) were analyzed. (*A*) Collagen synthesis was measured as nondialyzable [^{3}H]hydroxyproline and adjusted for the specific radioactivity of hydroxyproline in newly synthesized procollagen. (*B*) Total protein synthesis was measured as nondialyzable proline. Values are means±SEM (n = 5).

Table I. Effect of IFN-γ on Lung Fibroblast Collagen and Protein Synthesis

Culture conditions	[^{3}H]Hydroxyproline synthesis		Hydroxyproline-specific radioactivity	Hydroxyproline synthesis		
	Medium	Cell matrix		Medium	Cell matrix	Total
	dpm × 10^{-3}/μg DNA		*dpm/pmol*	*pmol/μg DNA*		
Control	4.68±0.16	1.67±0.08	39.7±2.6	118±4	42±2	160±6
IFN-γ	2.70±0.09*	1.42±0.06*	31.4±6.7‡	86±3*	45±2	131±5*
	[^{3}H]Proline incorporation			Proline incorporation		
	Medium	Cell layer		Medium	Cell layer	Total
	dpm × 10^{-4}/μg DNA			*pmol/μg DNA*		
Control	4.11±0.08	6.57±0.24		1,035±20	1,655±60	2,690±80
IFN-γ	2.62±0.09*	6.86±0.16		834±29*	2,185±51*	3,019±80*

IMR-90 fibroblasts were incubated under control culture conditions or with 1,000 U/ml IFN-γ for 72 h and then labeled with [^{3}H]proline for 6 h. After a 4-h chase period in isotope-free medium, the cell layers and labeling medium were analyzed as described in Methods. Values are means±SEM, $n = 5$. * $P < 0.01$, compared with control. ‡ $P < 0.05$, compared with control.

was due in part to IFN-γ-induced changes in the intracellular proline precursor pools, we directly measured the specific radioactivity of newly synthesized procollagen. The specific radioactivity was significantly lower in IFN-γ treated cultures (31.4±6.7 dpm/pmol in IFN-γ-exposed cultures vs. 39.7±2.6 dpm/pmol in control cultures). These values were used to determine actual hydroxyproline production (picomoles/microgram DNA) in the cultures. Exposure to IFN-γ resulted in a significant decrease in soluble newly synthesized procollagen in the culture medium. However, the amount of newly synthesized collagen deposited in the extracellular matrix was essentially the same in control and IFN-γ-exposed cell cultures. This relationship between matrix-associated collagen and total collagen is also reflected in the proportion of newly synthesized collagen incorporated into matrix; in control cultures 25.3% of the newly synthesized collagen was in the extracellular matrix, whereas in IFN-γ treated cells 34.2% was in matrix (Fig. 2). Total protein synthesis was assessed as [^{3}H]proline incorporation and corrected for specific radioactivity, assuming the same value as measured in newly synthesized hydroxyproline. Soluble protein secreted into culture medium was reduced by IFN-γ, but cell-associated protein was increased in IFN-γ-treated fibroblasts.

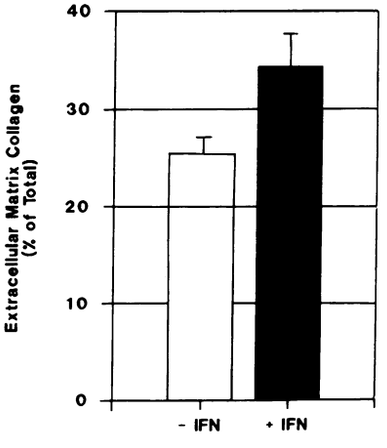

Figure 2. Effect IFN-γ on collagen matrix deposition. IMR-90 fibroblasts were cultured as described in Fig. 1. Nondialyzable [^{3}H]-hydroxyproline was measured in the medium and in cell layer–associated extracellular matrix. Values of [^{3}H]-hydroxyproline in the matrix are expressed as a percent of the total newly synthesized [^{3}H]-hydroxyproline. Values are means ±SEM ($n = 5$).

The effects of IFN-γ on collagen synthesis also were examined in cultures of human adult lung fibroblasts (2599N). The results were similar to those obtained with fetal lung fibroblasts (IMR-90). After IFN-γ exposure for 72 h, [^{3}H]hydroxyproline production by adult lung fibroblasts was decreased to 55% of control values ($2.13\pm0.22 \times 10^4$ dpm in IFN-γ-treated cultures vs. $3.87\pm0.86 \times 10^4$ dpm in control cultures, $P < 0.01$), and [^{3}H]proline production was reduced to 79% of control value ($2.86\pm0.42 \times 10^5$ dpm in IFN-γ-treated cultures vs. $3.98\pm0.86 \times 10^5$ dpm in control cultures, $P < 0.01$). The fraction of cell layer–associated [^{3}H]hydroxyproline increased from 11.4±1.4% in control cultures to 14.1±1.4% in IFN-γ-exposed cultures ($P < 0.05$).

To evaluate proteolytic processing of newly synthesized procollagen after IFN-γ exposure, the newly synthesized procollagen and processed forms in culture medium of IMR-90 fibroblasts were examined by SDS-PAGE. In control fibroblast cultures, procollagen and partially processed pC- and pN-collagens were the predominant components representing > 90% of the total collagenous proteins in medium. No change in the relative proportion of these components was observed after exposure to IFN-γ (data not shown), indicating that IFN-γ did not result in accumulation of steady-state levels of partially or fully processed soluble procollagen.

The human diploid lung fibroblasts examined in these experiments expressed a cell surface receptor for collagen that was recognized by a MAb and detected by indirect immunofluorescence labeling and analysis on a FACS. Fig. 3 shows a representative analysis of human diploid fetal lung fibroblasts (IMR-90) labeled with antibody to the collagen receptor (PIH5). The fluorescence intensity of fibroblasts labeled with PIH5 indicated strong positivity compared with fibroblasts labeled with an irrelevant antibody to a T cell-specific receptor or to unlabeled fibroblasts (cells and FITC-conjugate only). The intensity of fluorescence was comparable to that obtained by labeling cells with a class I MHC antibody (60.5), a cell surface antigen that is constitutively expressed on fibroblasts. The frequency distribution of cells labeled with antibody to the cell surface receptor for collagen indicated that essentially all fibroblasts in the population expressed the receptor.

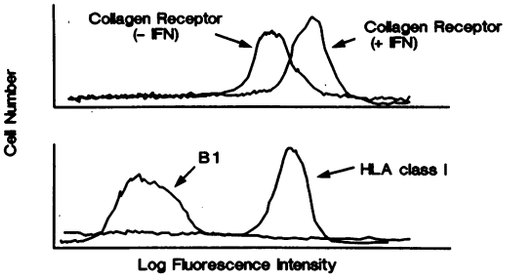

Figure 3. Fluorescence analysis of human diploid fibroblasts labeled by indirect immunofluorescence with antibody to cell surface receptors for collagen. Fibroblasts (IMR-90) were incubated under control conditions (−IFN) or with 1,000 U/ml IFN-γ for 72 h and labeled by indirect immunofluorescence and analyzed on a FACS as described in Methods. (*Top*) Analysis of cells labeled with antibody to cell surface receptors for collagen. (*Bottom*) Antibody specific for B lymphocytes (B-1), was used as a negative control, and antibody to a framework class I MHC antigen (HLA-I) was used as a positive control. Cells incubated with no antibody or fluorescein isothiocyanate–conjugated goat anti–mouse antibody (not shown) had a frequency distribution similar to that observed with B-1-labeled cells.

Analysis of indirect immunofluorescence for the cell surface receptor for collagen after exposure to IFN-γ demonstrated a significant increase in specific fluorescence intensity (Fig. 3) compared with fibroblasts grown under basal conditions, indicating increased receptor density on IFN-γ-exposed fibroblasts. The mean value for the log fluorescence intensity (expressed as mean channel number) was 151 in control cultures of IMR-90 fibroblasts, and 171 for IFN-γ-treated fibroblasts, representing a twofold increase in linear fluorescence intensity. The frequency distribution of labeled cells indicated that the fibroblast population was labeled in a log normal distribution. No discrete subpopulations with either comparatively weak or strong receptor expression were discovered. Similar shifts in fluorescence intensity were observed in IFN-γ-treated cells labeled with class I MHC antibodies (Table II). However, no increased expression of class II MHC receptors or increased expression in the unrelated receptor to platelet-derived growth factor was detected under these conditions. Thus, the increased expression of the collagen receptor does not appear to reflect a generalized or nonspecific induction of surface receptors. No difference in fluorescence activity was detected between cells detached with trypsin or EDTA (data not shown).

To evaluate the possibility that IFN-γ-induced increases in immunofluorescence were the result of increased cell size after IFN-γ, we examined fluorescence activity of control and IFN-γ-exposed fibroblasts as a function of cell size determined by forward light scatter. We found that the size distribution of cells was not changed by IFN-γ. As shown in Table III, IFN-γ-exposed cells exhibited greater immunofluorescence activity than comparable sized fibroblasts cultured under control conditions. Thus, the increased receptor expression is indicative of increased receptor density after IFN-γ exposure.

Similar patterns of fluorescence intensity and distribution were obtained with other human diploid lung cell strains (Table IV). Immunofluorescent intensity was similar in fibroblasts from both normal adult lung and fibrotic lungs. Moreover, no subpopulations with either comparatively weak or strong fluorescence positivity were discerned. Exposure to IFN-γ resulted in a 1.5–2.0-fold increase in linear fluorescence intensity, expressed as mean channel number, that had a log normal distribution about the mean.

Table II. Effect of IFN-γ on Expression of Cell Surface Receptors on Human Diploid Fibroblasts

Receptor*	Specific fluorescence intensity‡ (−)IFN-γ	(+)IFN-γ
Cells only	0	0
Control	1	1
B-1	2	2
Class I MHC	44	72 (320%)
Collagen	54	75 (210%)

Fibroblasts (IMR-90) were cultured under control conditions or with 1,000 U/ml IFN-γ for 72 h. Cell surface receptors were detected by indirect immunofluorescence labeling and analysis on a fluorescence activated cell sorter using a log fluorescence scale.

* See Methods for description of antibodies used for receptor labeling. Control cultures contained fluorescein isothiocyanate–conjugated anti–mouse IgG only. Antibody to a B lymphocyte receptor (B-1) also was used as a negative control.

‡ Specific fluorescence intensity is expressed as the difference between mean channel number of cells incubated with and without fluoresceinated IgG. The value given is the average of duplicate analyses. For each analysis, 4×10^3 cells were analyzed. The value in parentheses is the increase in linear fluorescence intensity expressed as a percent of the control value containing no IFN-γ.

Discussion

Cytokines derived from inflammatory cells may modify the biological responses of resident fibroblasts in lung and thus

*Table III. Effect of IFN-γ on Expression of Cell Surface Receptors for Collagen on Fibroblasts Separated by Forward Light Scatter**

Forward light window (Channel no.)	Specific fluorescence intensity −IFN	+IFN
1 (22–26)	53	71
2 (38–42)	66	80
3 (51–55)	73	83
	Cell number (%)	
1 (22–26)	946 (23.1%)	1057 (25.8%)
2 (38–44)	173 (4.2%)	134 (3.3%)
3 (51–54)	351 (8.6%)	298 (7.3%)

Fibroblasts (IMR-90) were cultured under control conditions or with 1,000 U/ml IFN-γ for 72 h. Cell surface receptors for collagen were detected by indirect immunofluorescence labeling and analysis on a FACS using a log fluorescence scale. Values of specific fluorescence intensity are expressed as the difference between mean channel number of cells incubated with and without fluoresceinated IgG. The value given is the average of duplicate analyses. For each analysis, 4×10^3 cells were analyzed.

Table IV. Effect of IFN-γ on Expression of Cell Surface Receptors for Collagen in Human Diploid Fibroblasts

Fibroblast strain	Specific fluorescence intensity (−)IFN-γ	(+)IFN-γ	Percent increase
Normal fetal lung (IMR-90)	73	84	162
Normal adult lung	30	42	172
Fibrotic adult lung (M.W.)	28	36	137
Fibrotic adult lung (G.N.)	35	45	155

Fibroblasts were cultured under control conditions or with 1,000 U/ml IFN-γ for 72 h. Cell surface receptors for collagen were detected by indirect immunofluorescence labeling and analysis on a FACS using a log fluorescence scale. Values of specific fluorescence intensity are expressed as the difference between mean channel number of cells incubated with and without fluoresceinated IgG. The value given is the average of duplicate analyses. For each analysis, 4 $\times 10^3$ cells were analyzed. The increase in receptor-specific fluorescence intensity on a linear scale is expressed as the percent of the control value.

modulate the remodeling of lung extracellular matrix in a variety of pathological conditions (4, 25, 34). However, the cytoregulatory effects of inflammatory cell mediators and the mechanisms by which they influence lung collagen accumulation are incompletely understood. In this study, we used cultured human diploid lung fibroblasts as a model system to examine the effects of recombinant IFN-γ on synthesis of collagen, matrix deposition of newly synthesized collagen, and the expression of cell surface receptors specific for collagen. The results indicate that synthesis of soluble collagen (procollagen) is decreased by IFN-γ while the proportion of newly synthesized collagen that is deposited into extracellular matrix is increased. An associated increase in fibroblast surface receptors for collagen was also observed, raising the possibility that IFN-γ modulation of this cell surface receptor may play a role in fibroblast collagen matrix formation. Previous studies have established a role for this collagen receptor in fibroblast adhesion to collagen matrices (18). In addition, this receptor (identical to platelet glycoprotein Ia-IIa complex) also mediates adhesion to collagen by platelets and leukocytes, cells that do not synthesize collagen, suggesting that involvement in cell adhesion may be its primary role (19). Modulation of the receptor and thus adhesive interaction of fibroblasts with collagen matrix could also therefore influence diverse cell responses including cell shape, orientation, and migration.

Increased expression of this cell surface receptor for collagen after exposure to IFN-γ was observed in normal fibroblast strains from both fetal and adult human lung, and in fibroblasts derived from lungs of two patients with idiopathic pulmonary fibrosis. Discrete subpopulations of fibroblasts with either greatly increased or decreased receptor expression were not identified in either the normal fibroblast strains or in the fibrotic lung fibroblasts strain although a range of receptor expression was identified by analysis of fluorescence intensity after indirect immunolabeling. These data indicate that expression of the receptor under control conditions or after IFN-γ exposure is not a property of a restricted subpopulation of cells as has been suggested for IFN-γ induction of Ia antigen (32). We found no evidence for altered expression of the receptor in fibroblasts cultured from fibrotic tissue, but this observation does not exclude the possibility that the phenotype of cells in fibrotic tissue is altered in situ. The receptor expression was enhanced by IFN-γ in all fibroblasts strains maintained in culture, suggesting that the presence of this effector molecule in inflammatory lung disease may similarly result in enhanced receptor expression in vivo.

Previous studies (23, 24) have focused on the direct inhibitory effect of IFN-γ on procollagen synthesis rates. Using a method that accounts for changes in proline precursor pool–specific radioactivity, we also found that IFN-γ reduced the total fibroblast collagen synthesis rate to 80% of control values. However, the amount of collagen deposited in the extracellular matrix was unaltered despite the decrease in total collagen synthesis rate in IFN-γ-exposed cells. Newly synthesized noncollagen protein, measured by incorporation of [^{3}H]proline, was also increased in the extracellular matrix after IFN-γ exposure. This observation is consistent with previous reports that the inhibitory effect of IFN-γ on dermal and synovial fibroblasts is selective for collagen (23, 32) and that production of specific noncollagen proteins (including fibronectin) may be induced by IFN-γ (38, 39).

The mechanism(s) that modulate the collagen deposition into matrix are not completely known. It is well established that collagen accumulation is a multistep process involving both intracellular and extracellular reactions. The fibroblast is involved in regulating synthesis of collagen and other matrix molecules and may modulate the extracellular processing of procollagen (1, 6–8, 37). It is less well established that fibroblasts exert control over other extracellular assembly events, but fibrillogenesis in tendon and cornea occurs within defined compartments determined by the fibroblast cell surface (9, 10). The recognition of a cell surface receptor specific for collagen (18) suggests that direct interaction of collagen with the cell surface can occur. The IFN-induced increase in cell surface receptors for collagen and the concomitant increase in collagen deposition observed in this study suggests that the receptors might directly influence matrix formation. Although involvement of the receptor in collagen matrix formation is speculative, upregulation of the related fibronectin receptor (22) has been suggested as a possible explanation for TGF-β-induced increases in the ability of fibroblasts to incorporate fibronectin into matrix (20, 22).

Several models for participation of the related fibronectin cell-adhesive receptor complex in matrix assembly have been suggested (40). Recent studies also suggest that a cell surface site, distinct from the cell adhesion receptor for fibronectin, mediates the assembly of exogenous fibronectin into fibrillar structures at the cell surface (21). It is also known that fibronectin is crucial to collagen matrix deposition in vitro (13, 41) and that fibronectin transcription and translation is increased by IFN-γ (39). These observations suggest the possibility that collagen matrix assembly might proceed in part as a consequence of interactions with fibronectin and/or a fibronectin receptor. Additional studies will be required to delineate the numerous possible steps in collagen matrix deposition. It may be informative to further examine the perturbation of in vitro matrix formation by cytokines as described in this study to learn more about these possible mechanisms.

Because of its inhibiting effect on collagen synthesis, a biological role for IFN-γ as a downregulating effector molecule has been postulated (23–25). However, IFN-γ has been shown

to enhance transcription of other matrix genes, including fibronectin (39). Moreover, our results indicate that deposition of newly synthesized collagen into matrix is preserved after IFN-γ exposure. Other recent studies have focused on IFN-γ's stimulatory function as an enhancer or inducer of class I and II MHC surface antigens as well as intercellular adhesion molecules (29, 31). Our results also demonstrate enhanced expression of a cell surface receptor for collagen. Thus, the effects of IFN-γ are multiple, and one of the important biological effects of IFN-γ may be secondary to the IFN-γ-induced expression of a surface receptor for extracellular collagen. Such an effect could modulate the interaction of fibroblasts with extracellular matrix at sites of inflammation and play an important role in the remodeling of matrix during repair from tissue injury.

Acknowledgments

The authors wish to thank Dr. David Coder, Director of the Flow Cytometry Laboratory at Fred Hutchinson Cancer Research Center, for helpful discussions in the preparation of this manuscript.

This work was supported in part by grant BC-419 from the American Cancer Society. Dr. Clark is the recipient of a Research Career Development Award (HL-01662) from the National Heart, Lung, and Blood Institute and a Career Investigator Award from the American Lung Association.

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