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Research Article

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Persistence of a Reduced-collagen-producing Phenotype in Cultured Scleroderma Fibroblasts after Short-term Exposure to Interferons

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Abstract

Transient exposure to inflammation-associated, fibroblast-stimulatory factors appears to initiate fibrosis by inducing the persistently activated phenotypes displayed by fibroblast cultures derived from scleroderma skin and other fibrotic tissues. To determine whether one class of fibroblast-inhibitory factors, the interferons (IFNs), plays a role in terminating fibrosis by acting as persistent fibroblast deactivators, we inhibited (40–60%) the growth and collagen production of normal dermal fibroblasts and hypercollagen-producing scleroderma fibroblasts by short-term exposure to IFN- α , β , or γ . During subsequent subculture in the absence of IFNs, the growth and collagen production of normal fibroblasts and the growth of scleroderma fibroblasts increased to untreated control levels after two to three passages. In contrast, collagen production by scleroderma fibroblasts remained inhibited for at least five passages (18 cell doublings) and was not further suppressed by subsequent IFN exposure. These data suggest that IFNs may help terminate fibrosis by suppressing persistently activated fibroblast functions.

Introduction

Fibroblasts are responsible for the production and maintenance of the connective-tissue matrix. Under basal conditions, the growth and synthetic activities of resident fibroblasts are of a limited nature and likely controlled only by interaction with native connective-tissue matrix components (1, 2). However, after either traumatic or pathological tissue injury, resident fibroblasts undergo a metabolic activation and exhibit the increased growth and synthetic rates required to effect the fibrotic repair of injured tissue. This fibroblast activation is believed to be controlled by the presence of numerous stimulatory factors released into the tissue fluid environment as a result of blood vessel disruption and the infiltration and activation of platelets, neutrophils, monocytes/macrophages, and T lymphocytes during the inflammation and granulation tissue phases of the fibrotic response (3–16).

Whereas fibrosis in response to traumatic injury is usually a transient process resulting in healed wounds with minimal scar formation, pathological fibrosis is often a persistent chronic process resulting in excessive tissue scarring. Cultured fibroblasts

derived from sites of pathological fibrosis, such as rheumatoid synovium, and keloid or scleroderma skin, display activated phenotypes characterized by increased production of the connective-tissue matrix components: collagens (17–23), glycosaminoglycans (17, 24), and fibronectin (21), as well as altered growth (17) and collagenase (18, 25) and prostaglandin E₂ production (25). Moreover, a portion of these cultured fibroblasts, activated by the in vivo environment from which they were excised, appear to be permanently, or at least persistently, activated as their hyperactive phenotypes persist through numerous subcultures in vitro (17–20, 22–25).

Studies investigating the environmental stimulatory factors responsible for inducing pathologic fibroblast activation have recently focused on those produced by mononuclear cells, because it has long been recognized that both T lymphocytes and monocytes/macrophages are present during the inflammatory phase of pathological fibrosis (26–29). These studies indicate that both T lymphocyte-derived lymphokines (15, 16, 29, 30) and monocyte/macrophage-derived monokines (12–14, 30–33) can stimulate normal fibroblast functions in vitro. Further, in two instances, namely, glycosaminoglycan and prostaglandin E₂ production, it has been demonstrated that the stimulated phenotypes induced by culture in lymphokine/monokine supernatants are retained in the absence of the stimulating agents and that the induced activated phenotypes persist throughout numerous cell doublings and subcultures (34, 35).

Mononuclear cell-derived factors may also play a role in moderating or in terminating the fibrotic response as lymphokines and monokines inhibiting the functions of both normal and activated fibroblasts have also been described (16, 23, 36–38). We and others have been recently identified those inhibiting fibroblast growth and collagen production as monocyte-derived interferon (IFN)¹- α/β and T lymphocyte-derived IFN- γ (39–41). However, in vitro studies of persistent phenotype deactivation have not yet been reported, and to this end, we have attempted to determine whether short-term exposure to IFNs can induce either normal dermal or in vivo-activated scleroderma-derived fibroblasts to persistently express a reduced-growth and/or a reduced-collagen-producing phenotype.

Methods

Interferons. Human recombinant DNA-derived (Hu-r) IFN- α_2 (SCH 30500; sp act, 1.2×10^8 U/mg of protein) from *Escherichia coli* was kindly supplied by Schering Corp., Kenilworth, NJ. Human naturally derived (Hu-n) IFN- β (1.4×10^6 U/mg) was obtained from Lee Biomolecular, Inc., San Diego CA. Hu-r *Escherichia coli*-derived IFN- γ (1.3×10^7 U/mg) was a gift from Genentech, Inc., South San Francisco, CA. The titer of these IFN preparations ranged from 6.6×10^7 to 5×10^4

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1. *Abbreviations used in this paper:* ATCC, American Type Culture Collection; DME, Dulbecco's modified Eagle's medium; Hu-n, human naturally derived; Hu-r, human recombinant DNA-derived; IFN, interferon.

U/ml, and after dilution they were added directly to fibroblast cultures to yield a final concentration of 1,000 U/ml.

Fibroblast cultures. Primary fibroblast cultures were initiated from full-thickness 4-mm punch biopsies using the explant method. The explants were cultured in 2-cm² wells (Linbro, Flow Laboratories, Inc., McLean, VA) in endotoxin-free Dulbecco's modified Eagle's medium (DME) containing 25 mM Hepes, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin plus 20% endotoxin-free fetal calf serum (FCS) (Whittaker-M.A. Bioproducts, Walkersville, MD) at 37°C in a 5% CO₂ humidified atmosphere. These primary fibroblast cultures grew to confluency in ~ 4 wk and were subsequently trypsinized and subcultured in DME-20% FCS. Nine normal dermal fibroblast lines were established from facial or mammary skin removed from seven women and two men (average age 44.2 yr) during cosmetic surgery. Three scleroderma fibroblast lines were derived from the clinically involved skin (two forearm, one hip-abdomen junction) of patients with progressive systemic sclerosis (scleroderma) of 1-5 yr duration. Histologic examination of these scleroderma skin specimens showed moderate fibrosis of the papillary and reticular dermis accompanied by perivascular mononuclear cell infiltration, indicating sampled skin was in the active sclerodematous stage of disease. Fibroblast lines derived from these biopsies were chosen for study because a previous investigation indicated that lines developed from active disease areas exhibit appreciably increased production of collagen (42). Two additional scleroderma fibroblast lines, EuKen (CRL 1108) and RoDas (CRL 1114), grown from explants of clinically involved scleroderma skin using culture conditions similar to ours (DME + 10% FCS) were obtained from the American Type Culture Collection (ATCC), Rockville, MD. The characteristics of one of these lines (EuKen) have been previously described (22). The five scleroderma skin donors (four women, one man; average age 45.0 yr) matched the normal donors for age and sex.

IFN treatment and subculture. Subconfluent fibroblast cultures were set up by placing 10,000 freshly trypsinized fibroblasts, contained in 1 ml of DME-20% FCS, into triplicate 2-cm² wells and incubating for 18 h at 37°C in a 5% CO₂ atmosphere to permit adherence to well bottoms. After adherence, medium was removed and replaced with 1 ml of fresh DME-20% FCS with or without 1,000 U/ml of either IFN-α₂, β, or γ, and cultures incubated for an additional 96 h. After 96 h of growth, fibroblasts were trypsinized, washed, and subcultured at 10,000 cells per 2-cm² well in the absence of IFNs. These subcultures were maintained without media changes and subsequently passaged every 7 d for an additional 4 wk during which time both untreated normal and untreated scleroderma fibroblasts underwent an average of 18 cell doublings. The growth and collagen production of untreated control and IFN-treated fibroblasts were assessed at each subculture as described below. After the last passage, fibroblasts were again tested for sensitivity to IFN treatment by incubation with 1,000 U/ml of IFN-α₂, β, or γ.

Assay of fibroblast growth. Assay of FCS-driven fibroblast growth was performed using asynchronous nonconfluent fibroblast cultures seeded at 10,000 cells per 2-cm² well as described above. Because FCS-driven fibroblast growth is caused by the orchestrated action of the numerous peptide growth factors contained in serum, we performed all the studies reported herein using the same lot of FCS in order to eliminate lot-to-lot variation in these peptide growth factors. After 96 h of culture in DME-20% FCS with or without IFNs, growth was assessed by counting the number of trypsin-released cells present using a hemocytometer. Assays were done using triplicate cultures and each culture was counted twice and values are expressed as the number of cells per 2-cm² well. Untreated control cultures reached ~ 80% confluency after 96 h of growth.

Assay of fibroblast collagen production. Assay of collagen production during a 24-h period by steady-state, confluent fibroblasts cultured in the presence of 10% FCS and ascorbic acid was assessed by [³H]proline incorporation into collagenous protein. The use of confluent cultures eliminates growth-related events; the use of 10% FCS insures optimal levels of collagen production (43), especially in scleroderma-derived fibroblasts (44) and may partially mimic the in vivo environment to which scleroderma fibroblasts are exposed during the edematous and sclero-

edematous stages of the disease, inasmuch as the microvascular endothelial cell damage and platelet activation associated with scleroderma would likely expose in situ scleroderma fibroblasts to the same growth and collagen-production stimulatory factors present in FCS (45). A single lot of FCS was used for all studies in order to eliminate variability owing to factors contained in FCS, such as transforming growth factor-β, which have collagen-production stimulatory activity (9, 30). In order to maximize the differences between normal and scleroderma fibroblast collagen production, we employed a suboptimal feeding schedule similar to those previously described (20, 23, 41), although other studies show that optimal feeding schedules will also reveal the hypercollagen-producing phenotype of scleroderma fibroblasts (22).

Fibroblasts grown to confluency after 7 d of culture without media changes were trypsinized and plated in triplicate in 96-well microcultures (Microtest III, Falcon Labware, Oxnard, CA) at a near-confluent density of 25,000 fibroblasts per well in 100 µl of DME-20% FCS and incubated for 48 h to produce a totally confluent monolayer of fibroblasts. The medium was then removed and replaced with 200 µl of DME plus 10% FCS containing 50 µg/ml ascorbic acid with or without IFNs, and incubated for an additional 48 h. Cultures were labeled with 0.5 µCi of [³H]proline (31 Ci/mmol; Amersham Corp, Arlington Heights, IL) and 50 µg/ml β-aminopropionitrile for the final 24 h of culture. The [³H]proline incorporation into pepsin-resistant, salt-precipitated extracellular collagen was then determined as previously described (37, 39, 46). In our hands this assay yields material that is 95% collagenase-digestible, and the method isolates < 1% of noncollagenous proteins biosynthetically labeled with [³H]tryptophan (39). Results are expressed as disintegrations per minute of [³H]collagen per 10³ cells with cell numbers determined by counting the number of trypsin-released cells present in a series of identically treated microcultures.

Results

Growth and collagen production of untreated control fibroblasts. The study was initiated with fibroblast lines in both the second and ninth passages. As shown in Table I, growth rates of both

Table I. Growth of Fibroblast Lines

Line no.	Fibroblasts	
	Normal	Scleroderma
	cells per 2-cm ² well	cells per 2-cm ² well
1	182,000*	98,000* [‡]
2	210,000*	102,000* [‡]
3	77,000*	84,000 [‡]
4	95,000*	72,000 [‡]
5	61,000 [‡]	132,000 [‡]
6	67,000 [‡]	
7	106,000 [‡]	
8	68,000 [‡]	
9	196,000 [‡]	
Average±SEM	118,000±20,000 [†]	97,000±10,000 [†]

The growth of asynchronous nonconfluent fibroblasts seeded at 10,000 per 2-cm² well growing in DME-20% FCS was assessed by counting the number of trypsin-released cells present after 4 d of growth (80% confluency). Each value is the mean of duplicate hemocytometer counts of triplicate cultures with standard deviation being ~ 10%.

* Fibroblast lines in ninth passage.

[‡] Fibroblast lines in second passage.

[‡] Scleroderma line 1 was EuKen and line 2 was RoDas.

[†] Averages are not statistically different ($P > 0.05$ by two-tailed t test).

normal and scleroderma lines at the initiation of the study without IFN treatment covered a two- to threefold range, but the average growth of the nine untreated normal and five untreated scleroderma lines (both in-house and ATCC-derived) was similar to that of 10,000 seeded cells expanding to ~ 100,000 cells over 4 d of growth as has previously been noted (19, 20, 22). During the 31-d, five-subculture course of the study, the growth rate of these untreated normal and scleroderma lines remained virtually constant with cells initiated in both the second and ninth passages (see Tables III and IV).

The collagen production of individual normal and scleroderma lines at the initiation of the study also varied over a two- to threefold range as illustrated in Table II. However, both in-house and ATCC-derived scleroderma lines exhibited an elevated collagen production, and the average collagen production of the five untreated scleroderma lines was 69% greater than the untreated normal cells, suggesting they possessed an activated collagen producing-phenotype as has previously been demonstrated in other studies (19–23). The collagen production of the untreated normal and scleroderma lines studied remained virtually constant over the 31 d of the study with the average scleroderma fibroblast collagen production remaining significantly greater than normal fibroblast production for the entire period, regardless of whether cultures were initiated in the second or ninth passage (see Tables V and VI).

Effect of short-term IFN treatment on fibroblast growth. To determine the effect of short-term IFN treatment on fibroblast growth and collagen production, fibroblasts were treated for 4 d with 1,000 U/ml of IFN- α_2 , β , or γ , and then the growth and collagen production of the fibroblasts subcultured in the absence of IFNs was followed for an additional five passages over 31 d as described in the Methods.

The mean effect of IFN treatment on the growth of nine normal fibroblast lines is shown in Table III. On day 0, after 4

Table II. Collagen Production of Fibroblast Lines

Line no.	³ H]Collagen	
	Normal fibroblasts	Scleroderma fibroblasts
	dpm/10 ³ cells	dpm/10 ³ cells
1	61*	148**
2	98*	245**
3	185*	136†
4	200*	141†
5	147‡	237‡
6	42‡	
7	74‡	
8	128‡	
9	69‡	
Average±SEM	112±18 [†]	181±24 [†]

The collagen produced during a 24-h period by steady-state, confluent, fibroblasts cultured in DME-10% FCS-ascorbate was assessed by [³H]proline incorporation into pepsin-resistant, salt-precipitated collagen as detailed in Methods. Each value is the mean of triplicate determinations with standard deviation being ~ 10%.

* Fibroblast lines in ninth passage.

‡ Fibroblast lines in second passage.

§ Scleroderma line 1 was EuKen and line 2 was RoDas.

[†] Averages are statistically different ($P < 0.05$ by two-tailed t test).

Table III. Normal Fibroblast Growth after IFN Treatment

Time posttreatment	Subculture posttreatment	Number of cells			
		Control	Hu-rIFN- α_2	Hu-nIFN- β	Hu-rIFN- γ
<i>d</i>		10^3 per 2-cm ² well±SEM			
0		118±20	59±12*	48±10*	60±12*
4	1	110±18	52±13*	48±19*	57±10*
10	2	107±25	79±18	76±20	70±13
17	3	89±18	85±17	90±8	83±16
24	4	107±3	100±12	105±10	109±15
31	5	116±17	120±9	118±9	123±8

Subconfluent cultures of nine normal dermal fibroblast lines were treated with IFNs (1,000 U/ml) for 96 h and then the growth of the fibroblasts subcultured in the absence of IFNs was followed for an additional five passages (18 cells doublings) over 31 d.

* Values are statistically different from untreated controls ($P < 0.05$ by two-tailed t test).

d of treatment with 1,000 U/ml of either rIFN- α_2 , nIFN- β , or rIFN- γ , cell growth was inhibited by 50–60% as we and others have previously reported (39, 47). However, after three subcultures (~ 10 cell doublings) in the absence of IFNs, cell growth returned to untreated control levels.

The growth of the five scleroderma fibroblast lines was similarly inhibited by 4 d of treatment with all IFNs as shown in Table IV. Similar to normal fibroblasts, the IFN inhibition of scleroderma fibroblast growth was also a transient effect, with all growth returning to untreated control levels after only three subcultures in the absence of IFNs.

Effect of short-term IFN treatment on fibroblast collagen production. The effect of IFN treatment on the collagen production of normal fibroblasts, shown in Table V, illustrates that on day 0 after 4 d of treatment, all three IFNs caused collagen production to be inhibited by 50–60% as we have previously reported (39). Once again, however, the IFN inhibition was only a transient effect, because collagen production returned to untreated control levels after two to three subcultures without IFNs.

In contrast, 4 d of treatment with all three IFNs not only initially inhibited the collagen production of scleroderma fibro-

Table IV. Scleroderma Fibroblast Growth after IFN Treatment

Time posttreatment	Subculture posttreatment	Number of cells			
		Control	Hu-rIFN- α_2	Hu-nIFN- β	Hu-rIFN- γ
<i>d</i>		10^3 per 2-cm ² well±SEM			
0		97±10	41±10*	45±12*	50±6*
4	1	100±14	46±13*	42±18*	51±13*
10	2	96±6	69±10*	58±25	54±19
17	3	97±14	95±5	86±14	92±17
24	4	105±21	102±7	100±5	90±8
31	5	94±19	96±13	95±15	96±4

Subconfluent cultures of five scleroderma fibroblast lines were treated with IFNs (1,000 U/ml) for 96 h and then the growth of the fibroblasts subcultured in the absence of IFNs was followed for an additional five passages (18 cell doublings) over 31 d.

* Values are statistically different from untreated controls ($P < 0.05$ by two-tailed t test).

Table V. Normal Fibroblast Collagen Production after IFN Treatment

Time posttreatment	Subculture posttreatment	³ H]Collagen			
		Control	Hu-rIFN- α_2	Hu-nIFN- β	Hu-rIFN- γ
<i>d</i>		<i>dpm/10³ cells\pmSEM</i>			
0		112 \pm 18	60 \pm 10*	48 \pm 12*	43 \pm 11*
4	1	106 \pm 16	59 \pm 11*	49 \pm 15*	42 \pm 15*
10	2	100 \pm 15	90 \pm 13	78 \pm 16	86 \pm 16
17	3	96 \pm 11	89 \pm 15	91 \pm 13	91 \pm 5
24	4	109 \pm 7	96 \pm 6	100 \pm 16	105 \pm 17
31	5	112 \pm 12	107 \pm 16	108 \pm 15	102 \pm 18

Subconfluent cultures of nine normal fibroblast lines were treated with IFNs (1,000 U/ml) for 96 h and then the collagen production of the fibroblasts subcultured in the absence of IFNs was followed for an additional five passages (18 cell doublings) over 31 d.

* Values are statistically different from untreated controls ($P < 0.05$ by two-tailed t test).

blast on day 0 by 50–60% but the collagen production of these cells, although rising somewhat through the first two passages, remained markedly reduced throughout the entire 31-d, 18-cell division, subculture period in the absence of IFNs (Table VI). Moreover, the reduced amount of collagen produced by these inhibited cells was now approximately equal to that produced by untreated normal fibroblasts. These data suggest IFNs have persistently reduced the collagen-producing phenotype of these scleroderma-derived fibroblasts.

Response of 31-d posttreatment fibroblasts to reexposure to IFNs. To determine whether these reduced-phenotype fibroblasts now behave as normal fibroblasts by responding to IFNs with a transient further reduction of collagen production, we reexposed 31-d posttreatment fibroblasts to the IFNs with which they had originally been treated. However, scleroderma fibroblast collagen production was now largely unresponsive to IFN treatment and was not markedly inhibited (Table VII). This was in contrast to the significant reinhibition of the growth of scleroderma fibro-

Table VI. Scleroderma Fibroblast Collagen Production after IFN Treatment

Time posttreatment	Subculture posttreatment	³ H]Collagen			
		Control	Hu-rIFN- α_2	Hu-nIFN- β	Hu-rIFN- γ
<i>d</i>		<i>dpm/10³ cells\pmSEM</i>			
0		181 \pm 24	82 \pm 13	74 \pm 14	56 \pm 9
4	1	166 \pm 11	81 \pm 7	72 \pm 13	50 \pm 9
10	2	174 \pm 13	106 \pm 8	98 \pm 7	101 \pm 8
17	3	169 \pm 20	104 \pm 14	103 \pm 15	108 \pm 11
24	4	160 \pm 19	104 \pm 16	101 \pm 16	98 \pm 13
31	5	155 \pm 14	105 \pm 14	95 \pm 13	85 \pm 11

Subconfluent cultures of five scleroderma fibroblast lines were treated with IFNs (1,000 U/ml) for 96 h and then the collagen production of the fibroblasts subcultured in the absence of IFNs was followed for an additional five passages (18 cell doublings) over 31 d. All IFN treatment values are statistically different from untreated controls ($P < 0.05$ by two-tailed t test).

Table VII. Response of 31-d Posttreatment Fibroblasts to Reexposure to IFNs

Fibroblast line	Treatment	Number of cells	³ H]Collagen
		<i>10³ per 2-cm² well\pmSEM</i>	<i>dpm/10³ cells\pmSEM</i>
Normal	Control	120 \pm 9	107 \pm 16
	Hu-rIFN- α_2	59 \pm 12*	62 \pm 13*
	Control	118 \pm 9	108 \pm 15
	Hu-nIFN- β	42 \pm 16*	61 \pm 12*
	Control	123 \pm 8	102 \pm 18
	Hu-rIFN- γ	65 \pm 9*	48 \pm 11*
Scleroderma	Control	96 \pm 13	105 \pm 14
	Hu-rIFN- α_2	40 \pm 13*	98 \pm 6
	Control	95 \pm 15	95 \pm 13
	Hu-nIFN- β	25 \pm 12*	91 \pm 16
	Control	96 \pm 4	85 \pm 11
	Hu-rIFN- γ	32 \pm 17*	70 \pm 14

Subconfluent cultures of nine normal and five scleroderma fibroblast lines were treated with IFNs (1,000 U/ml) for 96 h and then subcultured in the absence of IFNs for five passages over 28 d. Cultures of these fibroblasts, set up as described in Methods for assays of fibroblast growth or collagen production, were assayed for growth or collagen production on day 31 after incubation in the absence or presence of 1,000 U/ml of the IFNs to which they had originally been exposed.

* Values are statistically different from controls not reexposed to IFNs ($P < 0.05$ by two-tailed t test).

blasts and the growth and collagen production of normal fibroblasts. Thus, although IFN-treated scleroderma fibroblasts produce normal amounts of collagen, they do not seem to possess all the characteristics of normal fibroblasts inasmuch as their collagen production could not be reinhibited by subsequent IFN treatment.

Discussion

Because we had recently identified IFN- γ as the lymphokine and IFN- α and β as the monokines responsible for inhibiting fibroblast growth and collagen production, in this report we investigated whether IFNs could persistently normalize the activity of cultured fibroblast previously activated in vivo. We chose fibroblasts derived from scleroderma skin as our activated model because they display persistently activated phenotypes characterized by the hyperproduction of collagens, fibronectin, and glycosaminoglycans but normal growth and collagenase production rates (19–24). In this way we could study the effect of IFNs on both activated (collagen production) and normal (growth) functions in the same fibroblast line. Moreover, similar transcription-regulatory mechanisms may be responsible for the increased collagen production of scleroderma fibroblasts and the decreased collagen production of IFN-treated fibroblasts, inasmuch as scleroderma fibroblasts have increased levels of procollagen messenger RNA (mRNA), and IFN treatment decreases the procollagen mRNA levels of both scleroderma and normal fibroblasts (41, 48, 49).

The results of our study demonstrate that both scleroderma and normal fibroblasts are sensitive to the initial inhibitory effects of either IFN- α , β , or γ , inasmuch as the growth and collagen production of both fibroblast types were inhibited 40–60% by 96 h of IFN treatment. However, those fibroblast functions that were normally active, namely, the growth and collagen production of normal fibroblasts and the growth of scleroderma fibroblasts, returned to untreated levels during subsequent subculture in the absence of IFNs. In contrast, scleroderma fibroblast collagen production, an activated function, remained inhibited for 18 cell doublings in the absence of IFNs. This population of deactivated scleroderma fibroblasts, although producing normal amounts of collagen, was however, not identical to the normal unactivated fibroblast populations studied because its collagen production was no longer sensitive to IFN-mediated inhibition. These data suggest, that although IFNs are only transient inhibitors of normal fibroblast functions, they do indeed act as persistent deactivators of at least one activated fibroblast function. Whether IFNs inhibit other activated fibroblast functions such as glycosaminoglycan or fibronectin production in a persistent manner remains to be investigated. However, IFN- γ has been reported to inhibit both the collagen and fibronectin production of rheumatoid synovial fibroblast-like cells, although persistence of inhibition was not investigated (50).

In the present study, we have not attempted to analyze the types of collagens produced before and after IFN treatment nor the mechanism by which IFNs inhibit collagen production. However, previous reports demonstrate that in the presence of FCS both normal and scleroderma fibroblasts produce type I and III collagen in a ratio of $\sim 5:1$ (22), and short-term IFN treatment causes a coordinate decrease in the production of both type I and III collagen in normal and scleroderma fibroblasts as assessed by procollagen and procollagen mRNA levels (40, 41, 49). The IFN-induced decreases in procollagen mRNAs were proportionate to procollagen reduction and no detectable increase of collagen degradation products was observed, suggesting that IFNs act at a transcriptional level (40, 41, 49). Whether IFNs inhibit only the procollagen mRNA synthesis induced by the transforming growth factor- β contained in FCS-supplemented media (9, 39), or also inhibit basal level procollagen mRNA synthesis was not assessed. However, we have previously shown that IFNs or mononuclear cell supernatants containing IFNs can inhibit both normal and scleroderma fibroblast collagen production in cultures containing low (1%) or no FCS (37, 39, unpublished observations).

The mechanism responsible for the observed emergence of a persistently reduced collagen-producing phenotype after transient exposure of scleroderma fibroblasts to IFNs may involve either clonal selection or phenotype differentiation. Because the activated scleroderma fibroblasts that we studied were growing when treated with IFNs, it is possible that the deactivation of collagen production that we observed was due to clonal selection inasmuch as the IFNs were also growth inhibitors. Moreover, the collagen production of the deactivated scleroderma fibroblast was insensitive to subsequent IFN exposure, suggesting that a subpopulation of IFN-sensitive, high collagen-producing cells had been eliminated. Alternatively, growth-independent phenotypic differentiation with concurrent loss of sensitivity of collagen production to IFN treatment may have occurred because IFNs clearly inhibit collagen production in static, nongrowing cultures of both normal and scleroderma fibroblasts (39–41, 49). Such a theory is supported by our preliminary studies, which

demonstrate that when confluent, nongrowing cultures of two lines of scleroderma fibroblasts are transiently treated with IFNs and then maintained for 4 wk as confluent static cultures in 0.5% FCS (51), their collagen production is both persistently inhibited and insensitive to subsequent IFN treatment. Thus it is likely that alterations in cell growth are not required for the IFN-induced phenotype changes we have observed.

Having demonstrated that IFNs deactivate scleroderma fibroblasts it is logical for us to suggest that the excessive fibrosis seen in vivo in scleroderma may be due to the deficient in situ production of IFNs by infiltrating monocytes/macrophages and T lymphocytes during the early edematous and scleroedematous stages of disease (26–29). This, however, may not be the case, because peripheral blood mononuclear cells from scleroderma patients produce normal amounts of collagen production-inhibitory factors now known to be IFNs (23, 37). Production of excessive amounts of a collagen production-stimulatory lymphokine by scleroderma peripheral blood mononuclear cells has, however, been reported (52), and this factor may be responsible for in vivo activation of hypercollagen-producing fibroblasts. Nevertheless, our results suggest that scleroderma may be amenable to therapy with IFNs or IFN inducers in that increasing in vivo levels of IFN would likely block the effect of excessive stimulatory factors, resulting in fibroblast deactivation. Similarly, other diseases characterized by pathological fibrosis, such as forms of pulmonary and liver fibrosis, chronic graft-vs.-host disease, rheumatoid arthritis, localized scleroderma, keloids, and hypertrophic scars, may also benefit from IFN treatment, regardless of the mechanisms leading to aberrant fibroblast activation. This concept of general in vivo fibroblast deactivation by IFNs is supported by a recently reported study showing that in vivo administration of IFN- γ prevents accumulation of collagen in the lungs of bleomycin-treated mice (53) and by reports of delayed wound healing in viral infected mice that may be due to the presence of high levels of virally induced IFN (54).

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