γ INTERFERON IS THE LYMPHOKINE AND β INTERFERON THE MONOKINE RESPONSIBLE FOR INHIBITION OF FIBROBLAST COLLAGEN PRODUCTION AND LATE BUT NOT EARLY FIBROBLAST PROLIFERATION

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The in vivo observation that infiltrates of lymphocytes and monocytes are present in tissues undergoing both normal and pathological fibrosis has led to the in vitro investigation of the role played by mononuclear cells in regulating fibroblast functions (1–6). These studies indicate that activated T lymphocytes and monocytes/macrophages can effectively modulate several fibroblast functions through the release of soluble macromolecular factors, collectively categorized as lymphokines (LK)[1] and monokines (MK). LK and MK preparations have been found to have activities both as stimulators and inhibitors of fibroblast movement (7–9), proliferation (10–14), and protein production, including production of fibrosis-forming collagens (14–18). The relationships of these fibroblast-regulating factors to known immunoregulatory LK and MK are largely undetermined, except that studies indicate that interleukin 1 is the fibroblast growth–stimulating MK and fibronectin the fibroblast-chemotactic MK (11, 8).

We and others (12–15, 17, 18) have recently shown that human peripheral blood mononuclear cells activated with T cell mitogens, like concanavalin A (Con A), or monocyte activators, like lipopolysaccharide (LPS), produce, respectively, LK in the 50,000 M_r range and MK in the 20,000 M_r range that inhibit the growth and collagen production of cultured dermal fibroblasts. Since Con A is known to stimulate T lymphocytes to produce γ interferon (IFN-γ) (which has a reported gel filtration M_r of 50,000) and LPS stimulates monocytes/macrophages to produce IFN-α/β of 20,000 M_r, we investigated whether LK/MK inhibition of fibroblast proliferation or collagen production could be attributed to human IFN (19–21). Our results suggest that the LK and MK which inhibit fibroblast

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[1] *Abbreviations used in this paper:* DMEM, Dulbecco's minimum essential medium; Con A, concanavalin A: FCS, heat-inactivated fetal calf serum; IFN, interferon; LPS, lipopolysaccharide; LK, lymphokine; MK, monokine; PSS progressive systemic sclerosis; TCA, trichloroacetic acid.

collagen production are IFN-γ and IFN-β, respectively, and that those early acting LK and MK which inhibit fibroblast proliferation are not IFN.

Materials and Methods

Interferons. Human recombinant IFN-α_2 (SCH 30500; sp act, 1.2×10^8 U/mg protein) from *Escherichia coli* was kindly supplied by Schering Corporation, Kenilworth, NJ; human recombinant *E. coli*–derived IFN-γ (1.3×10^7 U/mg protein) was a gift from Genentech Inc., South San Francisco, CA. Research grade samples of naturally derived human IFN-α (2×10^6 U/mg) and IFN-γ (1×10^6 U/mg) were obtained from Meloy Laboratories, Inc., Springfield, VA. Naturally derived human IFN-β (2×10^5 U/mg) was obtained from Lee Biomolecular, Inc., San Diego, CA. The titer of these IFN samples ranged from 6.6×10^7 to 2.1×10^5 U/ml and they were added directly to fibroblast cultures after dilution to the desired concentrations with culture media.

Anti-IFN Antibodies. Polyclonal rabbit antibodies to human IFN-α were obtained from Meloy Laboratories (titer, 2×10^4 neutralizing U/ml) and Interferon Sciences, Inc., New Brunswick, NJ (1×10^5 U/ml). Polyclonal rabbit antibody to human IFN-β (5×10^4 U/ml) was from Lee Biomolecular, Inc. Both polyclonal goat and monoclonal murine antibodies to human IFN-γ (2×10^4 U/ml) were obtained from Meloy Laboratories, Inc. Neutralization studies were performed by incubating samples of LK and MK preparations with the desired concentration of anti-IFN antibody at 37°C for 1 h before being added to fibroblast cultures or submitted to antiviral assay.

Interferon Antiviral Assay. Analysis of the antiviral activity of LK and MK preparations was performed by Lee Biomolecular, Inc. using a cytopathic effect inhibition assay with diploid foreskin fibroblasts and encephalomyocarditis virus. The assay is a duplicate, serial endpoint quantitation and has a resolution of $\leq$30%. Antiviral activity was standardized against NIH human IFN-leukocyte and IFN-γ reference reagents and expressed as units per milliliter.

Production of LK and MK Supernatants Inhibiting Fibroblast Growth and Collagen Production. Supernatants were prepared from the human peripheral blood mononuclear cells recovered from Ficoll-Hypaque gradients as previously described (14). Briefly, mononuclear cells (2×10^6/ml) were incubated in endotoxin-free Dulbecco's minimum essential medium containing 25 mM Hepes and 2 mM glutamine (DMEM) (M. A. Bioproducts, Walkersville, MD) supplemented with 0.5% human serum albumin (Travenol Laboratories, Inc., Glendale, CA) and stimulated with 12.5 μg/ml Con A (Sigma Chemical Co., St. Louis, MO) or 20 μg/ml LPS (*E. coli* 0128:B12; Sigma Chemical Co.) to produce LK and MK supernatants, respectively. Cell-free supernatants were collected after 72 h incubation and exhaustively dialyzed against distilled water containing 0.01% thiodyglycol (Sigma Chemical Co.) before being dialyzed against fresh DMEM and assayed. Con A–induced supernatants were assayed in the presence of 25 mM α-methyl-D-mannoside (Sigma Chemical Co.).

Preparation of LK and MK Gel Filtration Fractions. Enriched LK and MK fractions of supernatants were prepared by gel filtration on Sephacryl S-200 (Pharmacia, Inc., Piscataway, NJ) eluting with phosphate-buffered saline containing 0.01% polyethylene glycol 6000 and 0.01% thiodyglycol, as described previously (14). LK activity eluted in the 50,000 M_r range and MK activity in the 20,000 M_r range.

Fibroblast Cultures. Primary cell cultures were established from skin explants taken from a normal neonatal foreskin and from a sample of normal adult facial skin removed during cosmetic surgery. The fibroblasts were grown to confluency in RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM glutamine, and 10% heat-inactivated fetal calf serum (FCS) (M. A. Bioproducts) and subcultured after trypsinization. Fibroblasts were used between the 3rd and 10th passages.

Assay for Activity Inhibiting Fibroblast Proliferation. Assay of fibroblast growth–inhibiting activity was performed using subconfluent fibroblast microcultures (Microtest III: Falcon Labware, Oxnard, CA) as previously described (14). Freshly trypsinized fibroblasts

were first plated at 3,000 fibroblasts per microwell in 100 μl of DMEM plus 10% FCS for 18 h at 37°C in a 5% CO_2 atmosphere to permit adherence to microwell bottoms. After 18 h adherence, medium was removed and dilutions of LK or MK preparations or IFN in 200 μl fresh DMEM plus 10% FCS were added to the fibroblast cultures for 24 h, along with 0.5 μCi of [^{3}H]thymidine (25 Ci/mmol; Amersham Corp., Arlington Heights, IL). After 24 h incubation, fibroblasts were harvested and [^{3}H]thymidine incorporation quantitated as reported (14). Briefly, after medium aspiration, cultures were trypsinized, harvested onto glass fiber filters using an automated harvester, and lysed by washing with distilled water. The [^{3}H]thymidine-labeled DNA that was trapped by the glass fiber filters was counted using liquid scintillation spectrometry. [^{3}H]Thymidine incorporation into DNA has been shown to be a true correlate of fibroblast growth by several investigators (10, 11, 14).

Assay for Activity Inhibiting Fibroblast Collagen Production. Trypsinized fibroblasts were plated at 20,000 fibroblasts per well in 100 μl DMEM plus 10% FCS for 18 h at 37°C in a 5% CO_2 atmosphere to produce a confluent monolayer of adherent fibroblasts, as described previously (14). After adherence, medium was removed and replaced with DMEM plus 1% FCS and incubated for an additional 48 h to allow fibroblasts to achieve proliferative quiescence. The medium was then removed and replaced with 200 μl fresh DMEM plus 1% FCS containing 50 μg/ml ascorbic acid and various dilutions of LK or MK preparations or IFN and incubated for an additional 48 h. Cultures were labeled with 0.5 μCi of [^{3}H]proline (31 Ci/mmol; Amersham Corp.) and 50 μg/ml β-aminopropionitrile for the final 24 h of culture. The [^{3}H]proline incorporation into pepsin-resistant, salt-precipitated extracellular collagen was determined as previously described (14, 22). The inclusion of β-aminopropionitrile ensures that >90% of the collagen produced will be secreted into the culture medium (23).

Assay for Activity Effecting Fibroblast Total Noncollagenous Protein Production. The effect of LK or MK preparations and IFN on noncollagenous protein production was assayed using culture conditions identical to those described for the assay of inhibition of collagen production, except fibroblasts were cultured in Ham's F10 medium which contains a low level of tryptophan, and labeled with 0.5 μCi [^{3}H]tryptophan (28.7 Ci/mmol; New England Nuclear, Boston, MA) instead of [^{3}H]proline, since tryptophan is not present in collagen (24). At the termination of culture, supernatant medium was removed from the fibroblast cell layer and the cell layer digested in 0.5 N NaOH. [^{3}H]Tryptophan-labeled protein contained in the cell digest and supernatant medium was precipitated with 7.5% trichloroacetic acid (TCA). The precipitates were collected onto glass fiber filters using an automated harvester, and washed, dried, and counted using liquid scintillation spectrometry.

Results

Titering and IFN Typing of Antiviral Activity Contained in LK and MK Preparations. Since the mode of induction and the M_r estimates of our fibroblast-inhibiting LK and MK suggested that they could be IFN, we determined the antiviral titers of our LK and MK preparations (Table I). A representative Con A–induced supernatant contained an antiviral titer equivalent to 1,000 U/ml of NIH reference IFN-γ, while an LPS-induced supernatant contained 2,300 U/ml. Moreover, neutralization of supernatant antiviral activity with specific anti-IFN antibodies indicated that Con A supernatants contained only IFN-γ and no IFN-α or -β. LPS supernatants contained primarily IFN-β, since anti-IFN-β almost entirely blocked antiviral activity. The partial block of LPS supernatant activity by anti-IFN-α is probably the artifactual result of the 30% accuracy limits of the assay, since the 20,000 M_r fractions that could contain both IFN-α and -β were only inhibited by anti-IFN-β. Con A supernatants thus contain IFN-γ, and LPS

TABLE I
Antiviral Titer and Type of IFN Contained in LK and MK Supernatants and Column Fractions

Sample	IFN Titer*			
		Neutralization with Anti-IFN‡		
		Anti-IFN-α	Anti-IFN-β	Anti-IFN-γ§
	U/ml		*U/ml*	
Con A–induced supernatant‖	1,000	1,000	1,000	<1.8
50,000 M_r fraction	330	250	250	<1.8
LPS-induced supernatant	2,300	1,500	16	2,300
20,000 M_r Fraction	450	480	10	420

* Titer listed is normalized to NIH human IFN-γ reference reagent.
‡ Samples neutralized with 2,000 neutralizing U/ml anti-IFN.
§ Anti-IFN-γ was a murine monoclonal antibody.
‖ Supernatants were assayed neat, and M_r fractions as 1:10 dilutions.

supernatants contain IFN-β, and chromatography provides no further enrichment for these IFN. Similar results were obtained when LK and MK preparations were referenced against an NIH human IFN-leukocyte reagent: LPS supernatant contained 290 U/ml and Con A supernatant, 180 U/ml. Unstimulated control supernatants contained <1.8 U/ml reference IFN-γ or <0.2 U/ml reference IFN-leukocyte. Thus, our LPS-induced MK preparations appear to contain principally IFN-β, and our Con A–induced LK preparations, IFN-γ.

Effect of IFN on Fibroblast Proliferation. IFN of all types and degrees of purity have been reported (25) to inhibit the proliferation of a variety of tumor and transformed cell lines, as well as normal cells in culture, including human fibroblasts. We investigated whether samples of natural and recombinant DNA–derived IFN-α, -β, and -γ could inhibit the proliferation of normal adult and neonatal dermal fibroblasts cultured under conditions optimal for growth inhibition by our LK and MK preparations (14). Under these subconfluent culture conditions (Fig. 1 and Table II), recombinant-derived IFN-α_2 and -γ at concentrations from 10 to 10^5 U/ml, and naturally derived IFN-β at 10 to 10^4 U/ml, did not inhibit normal fibroblast proliferation, while LK and MK preparations containing 1,000–2,000 U/ml IFN caused inhibitions of ≥50%. Samples of naturally derived IFN-α and -γ were also ineffective inhibitors of proliferation at 10 to 10^5 U/ml (data not shown). Previous studies (26) have also reported that IFN do not inhibit human fibroblast growth during the first 24 h of IFN treatment of asynchronized subconfluent cultures similar to those used here. Such cultures required 48–72 h of IFN treatment to demonstrate significant inhibition of growth (26). We also observed that at least 48–72 h of treatment was required to inhibit fibroblast growth. 96 h treatment with 10^3 U/ml of recombinant IFN-α_2 or -γ or natural IFN-β caused a growth inhibition of foreskin fibroblasts of 66, 56, and 57%, respectively. After 96 h of treatment, the growth of cultures treated with LK or MK preparations were still inhibited to the same degree as observed after 24 h of treatment (data not shown). These results suggest that our LK and MK preparations contained two growth-inhibiting

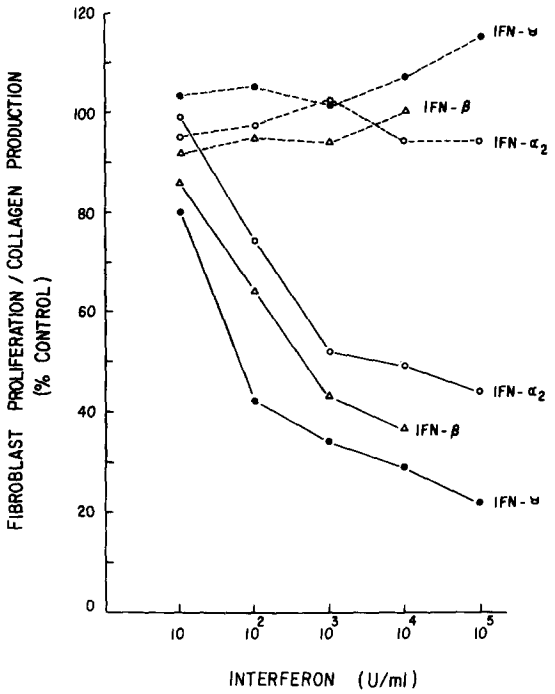

FIGURE 1. Concentration-dependent inhibition of foreskin fibroblast collagen production by IFN. Cultures of normal foreskin fibroblasts were incubated with increasing concentrations of recombinant DNA-derived IFN-α_2 (○), purified naturally derived IFN-β (△), or recombinant DNA–derived IFN-γ (●), and fibroblast proliferation (broken lines) and collagen production (solid lines) determined as described in Materials and Methods. Values are reported as a percentage of untreated control cultures (100%) and each point represents the mean of three separate experiments of triplicate determinations with interexperimental SEM being <10%.

components: the fibroblast growth–inhibiting LK and MK (14), which are early acting and not IFN, and IFN-γ and -β, which are late-acting inhibitors.

Effect of IFN on Fibroblast Collagen Production. Samples of natural and recombinant-derived IFN-α, -β, and -γ were assayed for inhibition of collagen production using fibroblast culture conditions similar to those we have previously determined (14) to be optimal for LK/MK-mediated inhibition of collagen production. Under these low serum, confluent culture conditions, stationary-phase fibroblasts are sensitive to agents that effect collagen production but unresponsive to growth-modulating signals. This permits the selective assessment of collagen production–inhibiting activity. As shown in Fig. 1 and Table II, recombinant IFN-α_2 and -γ and natural IFN-β caused concentration-dependent inhibitions of collagen production by both adult and neonatal fibroblast microcultures. Samples of naturally derived IFN-α and -γ gave inhibitions very similar to those shown for recombinant IFN-α_2 and -γ. At concentrations $\geq 10^3$ U/ml, all IFN samples inhibited fibroblast collagen production by ≥50%. These data, which present IFN strength in antiviral units per milliliter, suggest that IFN-α, -β, and -γ differ in potency as collagen production inhibitors. However, if IFN are compared on a molar basis, IFN-α, -β, and -γ are approximately equipotent inhibitors of collagen production. Molar comparison of IFN takes into account

TABLE II

Inhibition of Adult Dermal Fibroblast Collagen Production by IFN and LK and MK Preparations

	Units per milliliter	Inhibition of proliferation*	Inhibition of collagen production‡
None		8,708 ± 430	3,490 ± 171
Recombinant IFN-α_2	10^5	8,682 ± 726	1,472 ± 153
	10^4	8,676 ± 780	1,741 ± 201
	10^3	7,938 ± 141	1,841 ± 193
	10^2	7,548 ± 750	2,541 ± 212
Natural IFN-β	10^4	8,222 ± 472	1,285 ± 52
	10^3	7,830 ± 470	1,373 ± 161
	10^2	8,176 ± 482	2,275 ± 147
Recombinant IFN-γ	10^5	7,162 ± 978	897 ± 48
	10^4	7,511 ± 729	1,047 ± 153
	10^3	7,536 ± 558	1,254 ± 80
	10^2	7,602 ± 604	1,750 ± 98
Con A–induced supernatant§		3,047 ± 482	1,096 ± 44
50,000 M_r fraction		2,177 ± 381	1,211 ± 98
LPS-induced supernatant		3,982 ± 268	1,884 ± 109
20,000 M_r fraction		4,789 ± 511	2,078 ± 215

Representative experiment showing the effect of IFN and LK and MK preparations on the proliferation and collagen production of adult dermal fibroblasts cultured as described in Materials and Methods. Data was confirmed in one additional experiment.

* Counts per minute ± SD of triplicate determinations of [^{3}H]thymidine incorporation into DNA.

‡ Counts per minute ± SD of triplicate determinations of [^{3}H]proline incorporation into collagen.

§ Supernatants were assayed at a dilution of 1:2, and M_r fractions at 1:4.

the fact that the antiviral specific activity of pure IFN-γ is generally ~10-fold lower ($\cong 2 \times 10^7$ U/mg protein) than IFN-α or -β ($\cong 2 \times 10^8$ U/mg protein), and thus yields a more meaningful comparison of IFN collagen production–inhibiting activities. Together with the results in Table I, these findings suggest that IFN-γ is the component of our LK preparations that inhibits collagen production, while IFN-β is the inhibitor in our MK preparations. Furthermore, the magnitude of inhibition induced by the LK and MK preparations correlates with the antiviral IFN titer of each.

Effect of IFN and LK and MK Preparations on Total Noncollagenous Protein Production. To demonstrate that the observed inhibition of collagen production by IFN, LK, and MK was not due to a general toxic effect or a nonspecific suppression of total protein synthesis, we investigated the effect of these agents on the incorporation of [^{3}H]tryptophan into protein. [^{3}H]Tryptophan incorporation into TCA-precipitable material in a discriminating assay for total noncollagenous protein synthesis, since tryptophan is not present in collagen molecules (24). In an assay of parallel fibroblast cultures labeled with [^{3}H]tryptophan by

TCA precipitation or collagen assay (Materials and Methods), <1% of the [^{3}H]-tryptophan-labeled protein assayed as collagen. Neither IFN (10^2–10^5 U/ml) nor LK or MK preparations (Table III) inhibited the production of total noncollagenous proteins present in the cell layer and supernatant medium of adult dermal fibroblasts. Moreover, IFN-γ and LK preparations caused a marked increase in the production of total noncollagenous proteins in the cell layer and supernatant medium. The IFN-γ-induced increase may represent the induction of cell surface HLA-DR antigen and its shedding into the medium (27, 28). Results similar to those in Table III were also obtained using neonatal foreskin fibroblasts and naturally derived IFN-α and -γ (data not shown). Thus, inhibition of collagen production is not due to a general toxic effect of IFN, LK, or MK on fibroblast protein synthesis.

Effect of Anti-IFN on LK/MK-mediated Inhibition of Fibroblast Proliferation and Collagen Production. To further demonstrate that the collagen production–inhibiting activity of our LK and MK preparations was due to the IFN-γ content of our LK preparations and IFN-β in our MK preparations, we assayed LK and

TABLE III

Effect of IFN and LK and MK Preparations on Total Noncollagenous Protein Production by Adult Dermal Fibroblasts

	Units per milliliter	[^{3}H]Tryptophan incorporation	
		Media*	Cell layer‡
None		7,483 ± 806	35,791 ± 1,481
Recombinant IFN-α_2	10^5	6,884 ± 353	38,545 ± 5,029
	10^4	8,081 ± 241	42,765 ± 4,004
	10^3	7,931 ± 597	41,854 ± 2,735
	10^2	7,707 ± 973	42,192 ± 2,747
Natural IFN-β	10^4	6,330 ± 352	32,504 ± 2,194
	10^3	7,068 ± 176	39,250 ± 599
	10^2	7,019 ± 999	38,726 ± 3,535
Recombinant IFN-γ	10^5	20,378 ± 1,857	99,411 ± 10,713
	10^4	19,630 ± 662	89,413 ± 8,399
	10^3	17,478 ± 1,193	89,408 ± 3,583
	10^2	15,162 ± 1,133	74,127 ± 2,567
Con A–induced supernatant§		12,899 ± 1,523	76,198 ± 3,452
50,000 M_r fraction		14,257 ± 726	74,755 ± 3,447
LPS-induced supernatant		7,628 ± 1,487	39,770 ± 4,523
20,000 M_r fraction		7,879 ± 798	40,657 ± 5,335

Representative experiment showing the effect of IFN and LK and MK preparations on total noncollagenous protein production by confluent adult dermal fibroblasts cultured as described in Materials and Methods. Data was confirmed in one additional experiment.

* Counts per minute ± SD of triplicate determinations of [^{3}H]tryptophan-labeled protein precipitated from fibroblast culture media by TCA.

‡ Counts per minute ± SD of triplicate determinations of [^{3}H]tryptophan-labeled protein precipitated from digested fibroblast cell layer by TCA.

§ Supernatants were assayed at a dilution of 1:2, and M_r fractions at 1:4.

MK preparations for inhibiting activity in the presence of anti-IFN antibodies. The collagen production–inhibiting activity of Con A–induced supernatant and its 50,000 M_r fraction was completely suppressed (Table IV) by 10^3 neutralizing U/ml of monoclonal antibody to IFN-γ, while polyclonal antibodies to IFN-α and -β had no effect. A polyclonal anti-IFN-γ similarly suppressed LK collagen-inhibiting activity (data not shown). The collagen-inhibiting activity of LPS-induced MK supernatant and its 20,000 M_r fraction was suppressed by polyconal anti-IFN-β but not by anti-IFN-α or -γ. When tested alone, none of the anti-IFN significantly affected collagen production of control fibroblast cultures. These data confirm that collagen production–inhibiting LK and MK are IFN-γ and IFN-β, respectively. The data in Table IV also indicate that anti-IFN do not neutralize the proliferation-inhibiting activities exerted by LK and MK preparations after 24 h of treatment; thus, the early-acting growth-inhibiting LK and MK are not IFN.

TABLE IV

Selective Neutralization of LK and MK Inhibition of Foreskin Fibroblast Collagen Production by Anti-IFN

	Inhibition of proliferation*	Inhibition of collagen production*
Con A–induced supernatant‡	41 ± 8	42 ± 9
Plus anti-IFN-α§	36 ± 12	39 ± 4
Plus anti-IFN-β	47 ± 7	30 ± 9
Plus anti-IFN-γ	44 ± 3	102 ± 14
50,000 M_r fraction	30 ± 10	40 ± 3
Plus anti-IFN-α	37 ± 5	36 ± 7
Plus anti-IFN-β	30 ± 7	46 ± 16
Plus anti-IFN-γ	39 ± 8	94 ± 12
LPS-induced supernatant	54 ± 5	49 ± 6
Plus anti-IFN-α	60 ± 12	52 ± 3
Plus anti-IFN-β	47 ± 2	92 ± 10
Plus anti-IFN-γ	53 ± 9	63 ± 18
20,000 M_r fraction	43 ± 6	56 ± 9
Plus anti-IFN-α	40 ± 13	60 ± 11
Plus anti-IFN-β	48 ± 11	96 ± 8
Plus anti-IFN-γ	41 ± 4	58 ± 2

Data shows the effects of anti-IFN antibodies on the proliferation and collagen production–inhibiting activities of LK or MK preparations on foreskin fibroblasts as detailed in Materials and Methods.

* Values are reported as a percentage of untreated control cultures (100%) and each value represents the mean ± SEM of three separate experiments of triplicate determinations.

‡ Supernatants were used at a dilution of 1:2, and M_r fractions at 1:4.

§ Anti-IFN were used at a final concentration of 1,000 neutralizing U/ml.

Discussion

Fibrosis is a normal reparative process that occurs after tissue injury. In normal repairs, like wound healing, fibroblasts infiltrate the damaged area, proliferate, and synthesize the components of the connective tissue matrix, including proteoglycans and collagens. This restructuring of tissue with connective tissue results in scar formation, and fibrosis is terminated. Recent studies (7–18) indicate that mononuclear cells of the immune system may play an important role both in initiating and terminating fibrosis, through the release of LK and MK that stimulate or inhibit fibroblast functions. Of the LK and MK that may terminate fibrosis, those which inhibit fibroblast proliferation and collagen production have been the most extensively studied and characterized (12–15, 17, 18). Because these LK and MK share similar methods of induction and biochemical characteristics with IFN, we have investigated if these LK and MK inhibiting activities could be attributed to human IFN.

To ensure that our LK and MK preparations did indeed contain IFN, we first assayed them for antiviral activity and IFN type. The results (Table I) show the Con A–induced LK supernatants contained 1,000 U/ml of IFN-γ but no IFN-α or -β. LPS-induced MK supernatants contained almost 2,300 U/ml of, primarily, IFN-β, no IFN-γ, and perhaps low levels of IFN-α. The selective induction of T lymphocyte–derived IFN-γ by Con A and of macrophage-derived IFN-β by LPS have been previously reported (19, 20). Samples of purified, naturally derived IFN-α, -β, and -γ and recombinant DNA–derived IFN-α_2 and -γ were then assayed for inhibition of proliferation and collagen production by both neonatal and adult dermal fibroblasts.

We have previously shown (14) that LK and MK preparations inhibit the growth of nonconfluent, asynchronous fibroblast cultures after only 24 h of treatment. The tested IFN, however, caused no inhibition of fibroblast proliferation after 24 h of treatment and, in fact, required 48–72 h of treatment for significant inhibition of DNA synthesis. The delayed action of IFN was likely due to the fact that they do not directly inhibit DNA synthesis by cells in the S phase but prevent cells from entering the S-phase by blocking or prolonging the G_0/G_1 and G_2 phases (25, 29). The early action of LK and MK preparations suggests that they may directly inhibit DNA synthesis by cells in the S phase. This difference between the kinetics of LK/MK- and IFN-induced inhibition of fibroblast growth, and our additional experiments showing that anti-IFN antibodies do not block the early-acting, growth-inhibiting activities of LK or MK preparations, suggest that such activities are caused by factors distinct from IFN. The aberrant production of these early-acting, growth-inhibiting factors by patients with progressive systemic sclerosis (PSS) suggests they play an important role in terminating normal fibrosis (14).

IFN did mimic LK/MK action, however, by inhibiting the collagen production of confluent, steady-state fibroblast cultures. During the course of this study, others (30, 31) reported that purified, naturally derived IFN-α and -γ as well as recombinant-derived IFN-α_A and -γ inhibited fibroblast collagen production. Our results confirm and extend these reports to show that all types of IFN,

including IFN-β, inhibit collagen production. Moreover, our results demonstrate that the collagen production–inhibiting activity of LK preparations was selectively blocked by anti-IFN-γ and MK activity was blocked by anti-IFN-β, suggesting that collagen production-inhibiting LK and MK are IFN-γ and -β, respectively.

From the present study, it is impossible to determine the mechanism by which IFN inhibit collagen production, but a previous report (30) suggests that IFN-γ acts at the transcriptional level by inhibiting collagen mRNA synthesis. IFN-α and -β may act similarly but some studies suggest that they may be acting via increased prostaglandin production, since indomethacin blocks the effect of macrophage-derived (18) but not T lymphocyte–derived (17) inhibitors of collagen production.

Our results, identifying IFN-α, -β, and -γ as inhibitors of fibroblast collagen production and of late, but not early, fibroblast proliferation, suggest that IFN may be the mediators which terminate immunologically induced fibrosis. Further investigations are needed to determine the role IFN play in normal fibrotic repair and whether IFN production by mononuclear cells or IFN action on fibroblasts are altered in pathological fibrosis. Although IFN production and the action of purified or recombinant IFN on fibroblasts have yet to be studied in any fibrotic disease, the production and action of collagen production–inhibiting LK and MK have been studied in PSS. These studies show that normal levels of collagen production–inhibiting LK and MK (IFN-γ and -β) are produced by PSS patients (14) and that dermal fibroblasts grown from PSS subjects are sensitive to LK (IFN-γ)-mediated inhibition of collagen production (32). Although ruling out deficient IFN production as a causative factor in PSS, these studies suggest that PSS may be amenable to therapy with IFN or IFN inducers. Similarly, other diseases characterized by excessive fibrosis, such as idiopathic pulmonary fibrosis, liver cirrhosis, chronic graft-vs.-host disease, localized scleroderma, and keloid formation, may also be responsive to IFN treatment whether or not their etiologies involve decreased production of or response to IFN.

Summary

Human peripheral blood mononuclear cells activated with concanavalin A (Con A) or lipopolysaccharide (LPS) produce, respectively, lymphokines (LK) of 50,000 M_r or monokines (MK) of 20,000 M_r that inhibit the growth and collagen production of cultured human dermal fibroblasts. Because antigenic typing of the antiviral activity of these LK and MK preparations revealed that LK contained mainly γ interferon (IFN-γ), and MK, primarily IFN-β, we investigated if any of the fibroblast-inhibiting activities could be attributed to human IFN. Unlike LK and MK, which act within 24 h to inhibit the growth of subconfluent foreskin and adult dermal fibroblasts, samples of purified, natural derived IFN-α, -β, and -γ and recombinant DNA–derived IFN-α_2 and -γ were ineffective inhibitors at 24 h and required 48–72 h to significantly inhibit growth. However, all IFN did mimic LK/MK action in causing concentration-dependent inhibition of collagen production by confluent fibroblast microcultures. Furthermore, the collagen production–inhibiting activity of Con A–induced LK supernatant and its 50,000

M_r fraction was completely suppressed by 10^3 neutralizing U/ml of either polyclonal or monoclonal antibody to IFN-γ, while polyclonal antibodies to IFN-α and -β had no effect. Similarly, the collagen production–inhibiting activity of LPS-induced MK supernatant and its 20,000 M_r fraction was suppressed by polyclonal anti-IFN-β but not by anti-IFN-α or -γ. Anti-IFN failed to reverse early-acting LK or MK growth-inhibiting activities. These data suggest collagen production–inhibiting LK and MK are IFN-γ and IFN-β, respectively, and that early acting, growth-inhibiting LK and MK are not IFN.

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