Interferons and Collagen Production

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The immunoregulatory, antiviral, and antiproliferative agents known as the interferones have profound effects on collagen synthesis. Interferons α, β, and γ suppress collagen synthesis by dermal fibroblasts. In addition, interferon γ (IFN-γ) inhibits the constitutively increased collagen synthesis characteristic of fibroblasts derived from lesions of patients with scleroderma. IFN-γ also inhibits collagen synthesis by myofibroblasts and synovial fibroblast-like cells. Inhibition of collagen synthesis by IFN-γ is associated with a coordinate inhibition of transcription for type I and III collagen. In addition, IFN-γ suppresses levels of procollagen mRNA and type II collagen synthesis in human articular chondrocytes. In vivo studies in mice have demonstrated that IFN-γ inhibits the collagen synthesis associated with the fibrotic response to an implanted foreign body, bleomycin-induced pulmonary fibrosis, and the healing response to cutaneous thermal burns. In the latter case, while collagen content of the wound scar was decreased, hyaluronic acid was increased in mice receiving IFN-γ compared to controls. This is in accord with in vitro studies showing that, while interferons α and β decrease production of glycosaminoglycans, IFN-γ increases production of glycosaminoglycans. Of interest, acute inflammation at sites of thermal injury, or when elicited by proinflammatory agents in separate experiments, also was suppressed in mice treated with IFN-γ. The means by which IFN-γ inhibits collagen synthesis involves transcriptional regulation. There is a single report that interferon α can decrease the size of a keloid of recent onset in a human patient. Because the interferones can inhibit collagen synthesis in vivo, further studies may be warranted to evaluate the usefulness of these agents in the treatment of disease states characterized by abnormal fibrotic responses as well as their potential for altering the healing response associated with particular therapeutic interventions. J Invest Dermatol 95:755–808, 1990

Collagen is the primary structural protein of all tissues and is the major structural component of connective tissue. The synthesis and degradation of collagen normally is maintained under tight control but is altered in repair processes such as wound healing [1]. Pathologic fibrosis may occur as a result of abnormalities in collagen turnover in such diseases as hepatic fibrosis, pulmonary fibrosis, or, in the skin, morphea [2], scleroderma [3], or keloid formation [4-5]. Over the past decade, studies from a number of different investigators have demonstrated that products of inflammatory cells play a role in the regulation of collagen synthesis and degradation in cell culture systems [6-14]. These results led investigators to examine the effects of specific products of inflammatory cells on collagen synthesis at a molecular level, a cellular level, and in vivo. For example, interleukin 1, a cytokine produced by a number of cell types, increases collagen synthesis by synovial and dermal fibroblasts, murine mammary epithelial cells, and chondrocytes [14-16]. In contrast, the interferones inhibit production of collagen.

The interferones are a group of polypeptides which exhibit antiviral and antiproliferative effects (reviewed in [17]). They also have major immunoregulatory functions and have been shown to demonstrate some antitumor effects in vivo [17]. The interferones are a multigene family whose protein products have been divided into three main types. These are interferon α (IFN-α), interferon β (IFN-β), and interferon γ (IFN-γ) [17]. IFN-α was originally derived from peripheral blood mononuclear cells, IFN-β from fibroblasts, and IFN-γ from activated T lymphocytes and natural killer cells. The inhibition of collagen synthesis by these factors is the subject of this review.

In Vitro Studies

The ability of various interferones to affect collagen synthesis has been studied in vitro with a number of different target cell types. IFN-α has been demonstrated to inhibit collagen synthesis by human dermal fibroblasts [18,19]. Both purified and recombinant materials have this effect. Studies utilizing natural IFN-β demonstrated that this cytokine also inhibits collagen synthesis by human dermal fibroblasts [19].

Studies with IFN-γ have been performed with a wider variety of target cells. IFN-γ has been shown to decrease collagen synthesis by human dermal fibroblasts, human synovial fibroblast-like cells, human chondrocytes, rat myofibroblasts, and in fetal rat bone cultures [18-26]. Interestingly, it was reported that tumor necrosis factor α (TNFα) and IFN-γ are synergistic in their inhibition of collagen synthesis by human fibroblasts [27] and rat myofibroblasts [26].

Suppression of type I and type III collagen synthesis by dermal fibroblasts and synovial fibroblasts is associated with decreased levels of cellular mRNA for types I and III procollagen in these cells [20,21,23]. Similarly, suppression of type II collagen synthesis in cultured human articular and chondral chondrocytes by IFN-γ is associated with decreased levels of α1(I), α2(I), and α1(II) procollagen mRNA [24]. IFN-γ suppresses the level of α1(I) and α1(II) procollagen mRNA to a greater extent than α2(I) procollagen mRNA.
mRNA in articular but not in costal chondrocytes [24]. It is also of interest that the paper showing that TNFα is synergistic with IFN-γ in inhibiting collagen synthesis reported synergetism of these cytokines in inhibition of procollagen mRNA [27]. Thus, the regulation of collagen synthesis by IFN-γ appears to result in part from regulation at the level of transcription.

Studies have also indicated that IFN-α, IFN-β, and IFN-γ all reduce collagen production by fibroblasts derived from lesions of scleroderma, and, perhaps importantly, it was observed that this inhibition persists for at least 18 cell doublings after exposure [28–30]. Table I summarizes some of these observations.

In Vivo Studies In order to examine the in vivo and therapeutic relevance of the in vitro observations demonstrating that the interferons are capable of inhibiting collagen synthesis, a number of in vivo models of interferon actions have been examined. These studies were predominantly done utilizing IFN-γ.

In our laboratory, the ability of IFN-γ to alter collagen synthesis in a model of a fibrotic reaction to a foreign body in mice was initially examined [31]. In these experiments, small osmotic pumps (Model 2002, Alza Corp., Palo Alto, CA) were implanted subcutaneously into CAF, mice. We observed that within 14 d fibrous tissue developed and surrounded these pumps [31]. When assembled, these pumps measured 3.0 cm along the long axis and 0.7 cm in diameter. They have an outer membrane of cellulose ester and a cap at the exit port composed of ethylene polymer. Those employed in this study were capable of delivering 0.5 μl/h for 14 d.

We took advantage of the ability of these pumps to excrete solution for prolonged periods by examining the effects of IFN-γ on the fibrous capsule which develops around the pumps themselves [31]. Groups of animals were implanted with pumps loaded with recombinant murine IFN-γ (rMuIFN-γ) set to deliver 2,000 units/h or pumps loaded with diluent alone. Fourteen days later pumps were excised en bloc, and fibrous capsules surrounding each pump were carefully removed. Capsules surrounding pumps containing active rMuIFN-γ were thinner and smaller than those around control capsules. Figure 1 demonstrates this difference.

Additional experiments in this system examined the hydroxyproline content of the one-quarter of each capsule closest to the exit port of the pump in animals given pumps containing active rMuIFN-γ or diluent alone. The hydroxyproline content reflects the collagen content of these tissues. The mean hydroxyproline content per one-quarter capsule was reduced by an average of 87% in specimens taken from capsules loaded with active material compared to diluent control. Even after normalizing the hydroxyproline content of these specimens to dry weight of the tissue, a considerable difference is observed (63.5% reduction). Normalizing in this manner minimizes the difference in hydroxyproline content observed because much of the weight of specimens from either group is caused by collagen itself. An additional experiment compared the mean hydroxyproline content of specimens from animals given heat-treated (80°C for 30 min) rMuIFN-γ with specimens from diluent controls. In this experiment no significant difference was observed in the hydroxyproline content. One additional experiment was performed in which the hydroxyproline content of an entire capsule from a mouse given a pump containing active material was compared to a specimen from a mouse given a pump containing the diluent control. In this experiment the hydroxyproline content of the control specimen was 4.7 times greater than the rMuIFN-γ treated specimen.

Transmission electron microscopy was performed on specimens from capsules surrounding pumps loaded with rMuIFN-γ and from control capsules loaded with diluent alone. The capsules from control animals exhibited numerous thick collagen bundles, and fibroblasts contained endoplasmic reticulum dilated by granular material, an appearance consistent with active protein synthesis. In contrast, specimens from pumps containing active rMuIFN-γ showed thin collagen bundles within an electron lucent matrix and fibroblasts which did not show evidence of active protein synthesis. Computerized image analysis of randomly selected fields showed that collagen fibers from treated specimens occupied only 16.6% of the surface area occupied by fibers in control specimens.

The above experiments demonstrated a local ability of rMuIFN-γ to inhibit collagen synthesis in vivo. In order to examine the ability of IFN-γ to systemically alter collagen synthesis at distant sites, a skin wounding model was developed [32]. In this model, a pulse of radiation from an argon-ion laser (Model INNOVA 100–20, Coherent, Palo Alto, CA) operating in a single wavelength mode at 488 nm was utilized to produce fairly reproducible areas of full thickness coagulative necrosis of the skin. The effect of rMuIFN-γ on the healing of these wounds was assessed by administering this cytokine or control solutions with the same osmotic pumps utilized in the experiments above, but in this case implanted at a site distant from the skin lesion either subcutaneously or intraperitoneally [32]. In these experiments the pumps were loaded to deliver 8.7 × 10⁶ units/h for 14 d.

Figure 2 shows the gross appearance of these wounds 48 h after exposure to the laser. Figure 3 demonstrates the histologic appearance of these wounds. By 14 d after exposure to the laser, most wounds were healed in animals receiving active material as well as those receiving placebo. Figure 4 demonstrates the histologic difference between specimens obtained at 14 d from treated and control animals. Indeed, specimens obtained on days 10, 12, and 14 from animals that received IFN-γ had a decreased amount of fibrillar collagen compared to control animals. Fibers were of greatly

Table I. In Vitro Effects of Interferons on Collagen Synthesis

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<th>Interferon</th>
<th>Effects*</th>
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<tr>
<td>α</td>
<td>1. Inhibits collagen synthesis by human dermal fibroblasts.</td>
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<td>2. Reduces collagen production by scleroderma fibroblasts.</td>
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<tr>
<td>β</td>
<td>1. Inhibits collagen synthesis by human dermal fibroblasts.</td>
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<td></td>
<td>2. Reduces collagen production by scleroderma fibroblasts.</td>
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<tr>
<td>γ</td>
<td>1. Inhibits types I and III collagen synthesis by human dermal fibroblasts and synovial fibroblast-like cells.</td>
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<td></td>
<td>2. Inhibits type II collagen synthesis by human articular and chondral chondrocytes.</td>
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<td>3. Inhibits collagen synthesis by myofibroblasts.</td>
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<td></td>
<td>4. Inhibits collagen synthesis in fetal rat bone cultures.</td>
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<td></td>
<td>5. Reduces collagen production by scleroderma fibroblasts.</td>
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<td>6. Synergistic with TNFα in inhibiting collagen synthesis.</td>
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*From [18–27].
Transmission electron microscopy was performed on specimens from both day 14 and day 21. Quantitative image analysis of these specimens revealed a significant decrease in the percentage surface area composed of collagen bundles in treated as compared to control animals. At 14 d an average reduction of 36.5% was seen, and at 21 d a reduction of 40.7%. Furthermore, analysis of collagen fiber cross-sectional areas in day 21 specimens demonstrated a mean fiber cross-sectional of $9.4 \pm 0.48$ (SEM) $\mu m^2$ in the treated animals compared to a mean area of $21.43 \pm 1.02$ $\mu m^2$ in control wounds ($p < 0.001$). There was no significant difference, however, in the size of fibrils from these two groups.

Two other aspects of the effect of rMuIFN-γ on these healing wounds also deserve comment. First, it was noted that the time to healing of wounds, defined by the time to complete epithelialization as assessed grossly by a blinded observer, was prolonged in treated, compared to control, mice by 23% to 27% [32]. The other aspect was the unexpected finding that the acute inflammatory response to the wounding was reduced in treated as compared to control animals [32]. As shown in Fig 2, a greatly decreased perilesional erythema was noted in treated animals compared to control animals 48 h after wounding. Erythema in treated animals subsequently developed at 72 to 96 h, but never reached the intensity of that seen earlier in control animals. To obtain a quantitative measurement of the differences in blood flow between the perilesional areas in these two groups, a thermal camera was employed to measure the surface temperature of lesional areas at 48 h. A difference of $0.6^\circ C \pm 0.1$ (SEM) was observed. This corresponds to the differences seen grossly in perilesional erythema between treated and control mice and presumably relates to perilesional blood flow. In addition, histology of specimens from days 2, 4, 6, 8, and 10 demonstrated decreased polymorphonuclear cell infiltrate in perilesional areas in treated as compared to control animals on days 2, 4, and 6. By day 8, the difference was lost. In order to confirm this observation, the effect of rMuIFN-γ administered in this manner on inflammation induced by local injection of interleukin 1 or activated serum was examined. This was done by implanting pumps intraperitoneally to deliver rMuIFN-γ or diluent alone and injecting the left hind footpad of each mouse with 50 units of recombinant human interleukin 1 or a 1:2 dilution of Zymosan-activated serum 48 h later. Four hours later, animals were killed and footpad tissue prepared for histology. Using an ocular grid, cellular areas of

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**Figure 2.** Lesions 48 h after laser exposure. A: Lesion on mouse implanted with control pump. Note perilesional erythema. B: Lesion on mouse implanted with pump containing rMuIFN-γ.

reduced diameter and were decreased in density over the surface area of microscopic sections as compared to controls. In some experiments, groups of mice also received pumps containing heat-treated rMuIFN-γ (80°C for 30 min). Heat treatment abrogated the ability of rMuIFN-γ to alter wound healing. Because specimens from treated mice had increased space between collagen fibers, sections were stained for acid mucopolysaccharides with alcian blue (pH 2.5) with and without pretreatment with hyaluronidase. Specimens from rMuIFN-γ-treated animals contained more acid mucopolysaccharides than control specimens. In addition, pretreatment with hyaluronidase abolished the staining, suggesting that hyaluronic acid accounted for most of the additional acid mucopolysaccharides.

In an additional experiment, specimens were taken from animals on day 21, 7 d after the pumps were exhausted [32]. At this time, scar tissue from both groups, treated and control animals, was more mature. However, in the control specimen, thick horizontally oriented collagen bundles were evident, whereas specimens from treated animals showed smaller and less distinct collagen bundles.

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**Figure 3.** Murine skin 48 h after exposure to laser. Note focus of coagulase necrosis of epidermis, dermis, subcutaneous fat, and panniculus carnosus (magnification X 31).
Clinical Observations There is a recent report that IFN-α may be useful in the treatment of keloids when given as intralesional injections [35]. A keloid developed on a patient after receiving a small dose of carbon dioxide laser radiation. Ten weeks later, a small specimen of the lesion was surgically removed, and it was then injected with 1.5 million units of IFN-α2b. This dose of IFN-α2b was given again in the same manner 4 d later. This treatment resulted in a considerable decrease in size over the 9 d following the first injection. On the ninth day, an additional biopsy was obtained from the keloid. Following this, the lesion increased in size until at 30 d after the original biopsy, it was larger than at the time of the first injection. It was then injected 5 times over the next 10 d with the same dose of IFN-α2b, resulting again in a great reduction in size of the lesion. Keloidal fibroblasts obtained from the lesion before treatment produced more collagen, more glycosaminoglycans, and less collagenase than fibroblasts grown from the patient’s normal skin. In contrast, fibroblasts obtained from keloid specimens taken after treatment produced normal or subnormal amounts of collagen and glycosaminoglycans and normalized levels of collagenase activity.

This result is of great interest and suggests the possibility that the interferons may indeed be useful in fibrotic conditions of the skin. However, because this report is based on a single lesion in a single individual, further study is, of course, necessary to validate this result.

DISCUSSION

The observation that fibrous tissue undergoing repair processes or pathologic fibrosis contains various inflammatory and immunocompetent cells supports the concept that products of these cells may participate in the regulation of collagen synthesis and degradation. The experimental results that various cytokines can regulate the production of collagen by fibroblasts certainly support this concept. The experiments summarized above, in which the interferons are shown to inhibit collagen synthesis in vitro and in vivo, support the possibility of a physiologic role for these cytokines in the regulation of collagen synthesis. Additionally, they clearly suggest the possibility that such agents may be pharmacologically useful in the management of abnormal or inappropriate fibrosis.

Of additional interest was the observation that administration of IFN-γ is capable of decreasing acute inflammation as determined by decreased erythema, temperature, and acute inflammatory cell infiltration. These findings in some ways are surprising because IFN-γ has a number of activities that can be defined as proinflammatory, such as increasing the expression of intracellular adhesion molecule 1 [36], adherence of lymphocytes to endothelial cells [36], and binding of peripheral blood mononuclear cells and neutrophils to keratinocytes [37]. Nonetheless, the anti-inflammatory effects observed may be relevant to those reports that IFN-γ may be useful therapeutically in the treatment of patients with rheumatoid arthritis [38–42].

With regard to the possible therapeutic use of the interferons in fibrotic disease, it is noteworthy that IFN-γ in a number of studies appears to be more potent in suppressing collagen synthesis than IFN-α or IFN-β. On the other hand, the report that IFN-γ augments glycosaminoglycan production in vitro (supported by in vivo observations in the mouse), while IFN-α and IFN-β decrease glycosaminoglycan synthesis, might suggest that they may be more effective [44]. Only clinical trials of these agents will answer these questions. All three of these interferons reportedly increase collagenase release by fibroblasts in vitro modestly, but much less than agents such as interleukin 1 or tumor necrosis factor α [43]. The induction of collagenase activity may be important if the interferons are to be useful in reducing established fibrosis. Of particular interest is the report that TNFα and IFN-γ are synergetic in their suppression of
collagen synthesis and procollagen mRNA levels [26,27]. This observation obviously suggests the possibility that various combinations of cytokines used in conjunction may be more effective at suppression or induction of collagen synthesis than single agents. These observations set the stage for further research to define the optimum use of biologic response modifiers to achieve clinical goals.

REFERENCES

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