Collagen synthesis by human fibroblasts

Regulation by transforming growth factor- β in the presence of other inflammatory mediators

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We have examined the combined effects of transforming growth factor- β (TGF- β), serum and γ -interferon (γ -IFN) on collagen synthesis by fibroblasts and compared the response of fibroblast subpopulations to TGF- β . Human diploid fibroblasts were treated with TGF- β alone and with serum or γ -IFN. Cells were labelled with radioactive amino acids, and collagen production was measured as collagenase-digestible radioactivity. Collagen mRNA was determined by a solution-hybridization assay using procollagen- $\alpha 1$ [I] cDNA clone HF 677. The results showed that either serum or TGF- β increased incorporation, collagen production and mRNA by fibroblasts approx. 2-fold; however, collagen synthesis relative to total protein synthesis and collagen mRNA relative to total polyadenylated [poly (A)⁺] RNA were not affected. Only serum activated cell growth. Collagen production increased approx. 4-fold in cells exposed to both TGF- β and serum, and this increase was equal to that expected for an additive effect by both components. Treatment with γ -IFN decreased collagen production and collagen mRNA to 44 and 40 % respectively, whereas total incorporation and poly(A)⁺ RNA were affected only marginally. Cells exposed simultaneously to both γ -IFN and TGF- β produced less collagen and contained less mRNA than did those treated with TGF- β alone. The γ -IFN decreased collagen synthesis in control and TGF- β -treated cultures to a similar extent, and TGF- β increased collagen synthesis 2-fold in cells pre-treated with γ -IFN. Fibroblast strains obtained in medium containing plasma-derived serum synthesized approximately half as much collagen as did cells derived from the same explant in the presence of fresh human serum, and TGF- β stimulated collagen production and mRNA in both cell strains. We conclude that TGF- β , serum and γ -IFN regulate collagen synthesis by independent mechanisms, and that the combined action of these components plays a significant role in regulating collagen synthesis during wound healing and tissue repair.

INTRODUCTION

Wound healing and tissue repair involve a complex series of biological events which include inflammation, cellular migration, fibroblast proliferation, production of collagen and tissue remodelling (Ross, 1968; Dvorak, 1986). These events are influenced and regulated by the inflammatory response which followed immediately after tissue injury. A major component of inflammation is inflammatory cells, which infiltrate the damaged tissue to control invading micro-organisms and remove dead tissue. During this process, these cells are activated to secrete a variety of substances such as enzymes, growth factors and cytokines. Many of these substances affect the biological activities of fibroblasts and activate them to proliferate, migrate into the wound site and produce matrix constituents; once tissue integrity is restored, the numbers of fibroblasts and their synthetic activities return to normal values (Ross, 1968; Dvorak, 1986). Two factors will significantly influence the manner in which fibroblasts respond to the growth factors and other mediators of inflammation. First, several substances are present at the site of injury and some of them may have opposing effects. For example, platelet-derived growth factor and serum promote fibroblast growth, whereas products of activated mononuclear cells inhibit the growth (Korotzer *et al.*, 1982; Ross *et al.*, 1986). Serum, platelet-derived growth factor and TGF- β stimulate

fibroblasts to synthesize more collagen, whereas γ -IFN and mononuclear-cell factors cause inhibition (Narayanan & Page, 1983, 1987; Rosenbloom *et al.*, 1984; Narayanan *et al.*, 1985; Wrana *et al.*, 1986; Ignatz & Massagué, 1986; Ignatz *et al.*, 1987; Czaja *et al.*, 1987). Second, interactions between fibroblasts and regulatory molecules may be selective. Thus the C1 component of complement is mitogenic only to a subpopulation of fibroblasts, and prostaglandin E₂ and products of mononuclear cells affect growth and synthesis in only some, not all, fibroblasts (Ko *et al.*, 1977; Korotzer *et al.*, 1980; Korn *et al.*, 1984). Therefore, the types and concentrations of these molecules, which vary at different stages of inflammation, are likely to determine the progression of events from inflammation to repair, and aberrations of this process could affect normal wound healing and result in pathological conditions such as atherosclerosis and fibrosis (Ross, 1986). Because collagen synthesis is a major feature of wound healing, and fibrosis and TGF- β may play a significant role in these processes (Sporn *et al.*, 1983; Roberts *et al.*, 1986; Lawrence *et al.*, 1986; Mustoe *et al.*, 1987), we have studied how the stimulatory effect of TGF- β on collagen synthesis is affected by other substances which stimulate or inhibit collagen synthesis. We have used serum and γ -IFN respectively to mimic these effects (Ross, 1968; Rosenbloom *et al.*, 1984; Narayanan & Page, 1987;

Abbreviations used: TGF- β , transforming growth factor- β ; γ -IFN, γ -interferon; poly(A)⁺ RNA, polyadenylated RNA.

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Czaja *et al.*, 1987). In addition, we have also examined how TGF- β affects collagen production in two sub-strains of fibroblasts which were obtained from the same source but differ in growth rate, cell-surface receptors and capacity to produce collagen. The last case is particularly illustrative of healthy and diseased tissues which appear to contain different fibroblast subtypes that may not respond to regulatory molecules in the same manner (LeRoy, 1972; Hassell *et al.*, 1976; Bordin *et al.*, 1984; Korn, 1985).

EXPERIMENTAL

Materials

TGF- β was generously given by Dr. M. B. Sporn, N.C.I., Bethesda, MD, U.S.A. Recombinant γ -IFN (sp. activity 10^7 units/mg of protein) was a product of AMGen Biologicals, Thousand Oaks, CA, U.S.A. Human procollagen- $\alpha 1$ [I] clone HF677 cloned into Riboprobe pSP65 vector was kindly provided by Dr. Paul Bornstein, Department of Biochemistry, University of Washington. Radioactive amino acids and nucleotides were obtained from New England Nuclear, Boston, MA, U.S.A. Purified bacterial collagenase was a product of Advance Biofactures Corp., Lynbrook, NY, U.S.A.

Fibroblast culture and labelling

Fibroblasts were obtained from explants of human gingiva taken from individuals with clinically and radiographically healthy periodontal tissues. Cells were grown and maintained as described previously (Narayanan & Page, 1983). Each experiment was done in triplicate and repeated at least three times. Cultures of subconfluent cell density (1.6 ± 10^4 cells/cm²) were treated with the substances specified in Dulbecco-Vogt medium for 24 h. Before labelling, cells were preincubated for 1 h in serum-free medium without the mediators but containing 50 μ g each of ascorbic acid and β -aminopropionitrile/ml (Hassell *et al.*, 1976; Ko *et al.*, 1977). They were then labelled for 3 h with L-[2,3-³H]proline (sp. radioactivity 35.2 Ci/mmol) and [2-³H]glycine (sp. radioactivity 43.5 Ci/mmol) (each 10 μ Ci/ml) (Narayanan & Page, 1983, 1987).

Measurement of incorporation and collagen production

After labelling, medium was separated and cells were harvested in 0.5 M-NH₄OH. Medium and cell proteins were pooled and proteins were precipitated with 10% (w/v) trichloroacetic acid containing 1% tannic acid. The precipitate was washed and incorporation measured as described previously (Huey *et al.*, 1980). Collagen was quantified after digestion with purified bacterial collagenase (Huey *et al.*, 1980).

RNA extraction and determination of mRNA

Total fibroblast RNA was extracted by the guanidine isothiocyanate/CsCl gradient procedure of Chirgwin *et al.* (1979). Procollagen mRNA was determined in terms of procollagen- $\alpha 1$ [I]mRNA by a solution-hybridization procedure described by Voss & Bornstein (1986). The RNA probe used was prepared by labelling pro $\alpha 1$ [I] clone HF 677, which was subcloned into pUC12 polylinker site downstream to a SP6 promotor, with 5'-[α -³²S]thio]UTP (sp. radioactivity 1400 Ci/mmol) (Voss & Bornstein, 1986). Pro $\alpha 1$ [I] RNA standard was from the same plasmid in which the cDNA was linked in the opposite orientation (Voss & Bornstein, 1986). Various concentrations of RNA from each culture, ranging from 0.25 to 2.0 μ g, were hybridized with the probe at 68 °C for 16 h, after which unhybridized material was digested with a mixture of RNAases A and T₁. Then the RNA hybrid was precipitated with ice-cold 10% trichloroacetic acid, filtered and counted for radioactivity (Narayanan *et al.*, 1985; Voss & Bornstein, 1986; Narayanan & Page, 1987). Poly(A)⁺ mRNA was determined similarly, except that hybridization was done for 3 h each at 65 °C and then 37 °C (Voss & Bornstein, 1986).

Measurement of fibroblast proliferation

Approx. 2.5×10^3 cells were seeded in Linbro wells. After the cells attached to the wells, medium was replaced with fresh medium containing 0.5% serum, 0.5% serum plus 40 pM- or 200 pM-TGF- β or 10% serum with or without 40 pM- or 200 pM-TGF- β . Triplicate cultures were treated with trypsin at 2-day intervals, and cell number was determined with a Coulter counter.

Table 1. Collagen synthesis by human fibroblasts in the presence of serum and TGF- β

Values for collagen are means $\pm$ s.d. for triplicate samples. 'Ratio' is the +TGF- β /-TGF- β ratio. Values in parentheses are means $\pm$ s.d. for four (production) or three (mRNA) separate experiments. Abbreviation: ND, not done.

Serum	TGF- β	Collagen (d.p.m./100 cells)	Ratio	Collagen mRNA (fg/cell)	Ratio
—	—	2.33 $\pm$ 0.76	—	2.9	—
—	+	5.78 $\pm$ 1.09	2.5 (2.0 $\pm$ 0.3)	5.4	1.9 (1.8 $\pm$ 0.1)
+ 1%	—	3.21 $\pm$ 0.19*	—	ND	—
+ 1%	+	6.46 $\pm$ 1.25	2.0 (2.2 $\pm$ 0.6)	ND	—
+ 10%	—	3.99 $\pm$ 0.96*	—	5.3*	—
+ 10%	+	8.76 $\pm$ 0.74†	2.2 (2.0 $\pm$ 0.4)	8.9†	1.7 (1.7 $\pm$ 0.1)

* Increase in collagen production in the presence of 1 and 10% serum relative to 0% serum was 1.4- and 1.7-fold respectively; 10% serum caused a 1.8-fold increase in collagen mRNA.

† 3.8 (3.7 $\pm$ 0.6)- and 3.1 (3.0 $\pm$ 0.1)-fold relative to the -serum/TGF- β treatment.

Fig. 1. Effect of TGF- β and serum on the proliferation of human fibroblasts

Cells were plated at sub-confluent density in Linbro wells and exposed to 0.5% serum alone ($\circ$ — $\circ$), or 0.5% serum plus 40 pM- ($\circ$ — $\circ$) or 200 pM- ($\circ$... $\circ$) TGF- β , or 10% serum alone ($\bullet$ — $\bullet$) and 10% serum plus 40 pM- ($\bullet$ — $\bullet$) or 200 pM- ($\bullet$... $\bullet$) TGF- β . At 2-day intervals cells were harvested with trypsin and cell numbers were determined. The results plotted are means $\pm$ S.D. of three plates for each treatment.

Statistical significance

This was determined by Student's *t* test.

RESULTS

Effect of TGF- β on serum stimulation of collagen synthesis

To detect potential interactions between serum and TGF- β and to determine whether their effects on collagen synthesis are mutually exclusive, additive or synergistic, fibroblasts were incubated for 24 h in medium supplemented with 1% and 10% fetal bovine serum alone or with 40 pM (1.0 ng/ml) TGF- β [this concentration of TGF- β caused near-maximal stimulation of collagen synthesis in our experiments (results not shown) and elsewhere (Wrana *et al.*, 1986)], and collagen production and mRNA were measured. Collagen production increased 1.4- and 1.7-fold in the presence of 1% and 10% serum respectively (Table 1). TGF- β caused a 2.5-fold increase in collagen production in the absence of serum (2.0 ± 0.3 -fold in four separate experiments); in the presence of 1% and 10% serum, the enhancement was 2.0- and 2.2-fold (2.2 ± 0.6 and 2.0 ± 0.4 , $n = 4$) respectively. When both 10% serum and TGF- β were present in cultures, collagen production was 3.8 (3.7 ± 0.6)-fold as much as those containing no serum or TGF- β (Table 1). Collagen mRNA increased 1.8 ± 0.1 - and 1.7 ± 0.1 -fold ($n = 3$) in the presence of TGF- β plus 0 and 10% serum

respectively. The increase in presence of both TGF- β and 10% serum, relative to cultures without them, was 3.1 ± 0.1 -fold. The stimulation of collagen production and mRNA by TGF- β plus serum was significantly greater than that observed with either component alone ($P < 0.005$). Similar results were obtained for the incorporation of label into total proteins. The total incorporation increased 1.8 ± 0.4 - and 1.6 ± 0.3 -fold ($n = 4$) in the presence of TGF- β plus 0 and 10% serum respectively, and corresponding values for poly-(A)⁺ mRNA were 1.9 ± 0.3 - and 2.0 ± 0.1 -fold ($n = 3$) respectively (results not shown).

Because both serum and TGF- β (under some conditions; Centrella *et al.* 1987) are mitogenic to fibroblasts, we examined whether the stimulation of collagen synthesis by these two components is related to their effect on cell proliferation. However, although the cell number increased in the presence of 10% serum, TGF- β had no significant effect, with either 0.5% or 10% serum (Fig. 1).

Effect of TGF- β on γ -IFN inhibition of collagen synthesis

We performed three experiments to determine how both γ -IFN and TGF- β affect collagen synthesis together. In the first experiment collagen production and mRNA contents of fibroblast cultures exposed to 250 units of γ -IFN/ml (this concentration caused maximal inhibition of collagen synthesis; results not shown) plus 40 pM-TGF- β were measured. γ -IFN decreased collagen production to $44 \pm 12\%$ ($n = 6$) and mRNA to $40 \pm 19\%$ ($n = 3$) (Table 2). Cultures containing both γ -IFN and TGF- β produced greater amounts of collagen and mRNA than did those treated with γ -IFN alone. However, they were less than those exposed to only TGF- β (Table 2), and this did not increase even with 200 pM-TGF- β (results not shown). Collagen production and mRNA by these cultures were decreased to $62 \pm 8\%$ and $48 \pm 23\%$ respectively, and the inhibition (in the presence of TGF- β) was comparable with that observed with γ -IFN alone (Table 2).

In contrast with collagen, total incorporation was affected only marginally by γ -IFN; it was decreased to $89 \pm 25\%$ ($n = 6$) and $83 \pm 14\%$ ($n = 3$) in the presence of γ -IFN alone and plus TGF- β respectively (results not shown). Corresponding values for poly(A)⁺ mRNA were $97 \pm 1\%$ and $125 \pm 5\%$ respectively, indicating no significant inhibition.

In the next experiment we examined whether addition of TGF- β reverses the inhibition in cells preconditioned with γ -IFN. Fibroblasts were treated with γ -IFN for 24 h, then the medium was removed and cells were washed. Fresh medium alone or with TGF- β or γ -IFN was added, and after 24 h collagen production and mRNA were measured. These increased approx. 2-fold in the presence of TGF- β (Table 3), and this increase was not significantly different from that observed with TGF- β alone ($P > 0.4$). However, they were not restored to the values of untreated controls (Table 3), even with 200 pM-TGF- β (results not shown).

The effect of γ -IFN on cultures previously exposed to TGF- β was then examined. Cells were first incubated with TGF- β for 24 h, medium was removed and cells were washed. Incubation was continued for a further 24 h with fresh medium or medium supplemented with γ -IFN or TGF- β . Collagen production and mRNA

Table 2. Effect of TGF- β and γ -IFN on collagen synthesis by human fibroblasts

γ -IFN was added in serum-free medium. Values for collagen are means $\pm$ S.D. for three plates.

TGF- β	γ -IFN	Collagen		mRNA	
		(d.p.m./100 cells)	(%)	(fg/cell)	(%)
—	—	6.36 $\pm$ 0.30	100	2.4	100
—	+	3.24 $\pm$ 0.52	51 (44 $\pm$ 12*)	0.6	25 (40 $\pm$ 19*)
+	—	14.46 $\pm$ 1.92	100†	4.2	100†
+	+	8.16 $\pm$ 0.94	56 (62 $\pm$ 8‡)	1.4	33 (48 $\pm$ 22‡)

* Mean $\pm$ S.D. for six separate experiments.

† Collagen production and mRNA relative to untreated cultures were 227 % (241 $\pm$ 54) and 223 % (191 $\pm$ 16) respectively.

‡ Mean $\pm$ S.D. for three separate experiments.

Table 3. Effect of TGF- β on collagen synthesis by human fibroblasts previously treated with γ -IFN

Cells were treated with γ -IFN in serum-free medium for 24 h after which medium was removed and cells were washed. Fresh medium supplemented with the specified polypeptides was added, and the cells were incubated for a further 24 h. Collagen production and mRNA were then measured. Values for collagen are means $\pm$ S.D. for triplicate samples. Values in parentheses are means $\pm$ S.D. for three separate experiments. Differences between these and the % increase obtained with TGF- β alone were not statistically significant ($P > 0.4$). 'Control' refers to cultures not treated with either γ -IFN or TGF- β .

Addition	Collagen		mRNA	
	(d.p.m./100 cells)	(%)	(fg/cell)	(%)
None	1.14 $\pm$ 0.35	100	1.0	100
γ -IFN	1.21 $\pm$ 0.15	—	1.3	—
TGF- β	2.03 $\pm$ 0.10	178 (241 $\pm$ 79)	2.3	230 (186 $\pm$ 34)
Control	6.37 $\pm$ 0.3	—	4.4	—

Table 4. Effect of γ -IFN on collagen production by human fibroblasts previously treated with TGF- β

Cultures were treated with 40 pM-TGF- β for 24 h. Medium was then removed, cells were washed and fresh serum-free medium containing the substances specified was added. The incubation was continued for 24 h and then collagen production and mRNA were measured. Values for collagen are means $\pm$ S.D. for triplicate samples. Values in parentheses represent means $\pm$ S.D. for three separate experiments. The differences between TGF- β / γ -IFN and control/ γ -IFN treatments were not statistically significant ($P > 0.2$ and > 0.5 for collagen production and mRNA respectively). The 'control' cultures were not subjected to the first TGF- β treatment.

Addition	Collagen		mRNA	
	(d.p.m./100 cells)	(%)	(fg/cell)	(%)
None	3.09 $\pm$ 1.72	100	8.8	100
γ -IFN	1.18 $\pm$ 0.15	38 (55 $\pm$ 14)	5.0	42 (38 $\pm$ 24)
TGF- β	2.76 $\pm$ 0.02	—	9.0	—
Control, none	1.59 $\pm$ 0.12	100	2.9	100
Control, γ -IFN	0.52 $\pm$ 0.09	33 (45 $\pm$ 12)	0.8	47 (40 $\pm$ 19)

were decreased to 55 $\pm$ 14 and 38 $\pm$ 24 % respectively in cultures exposed to γ -IFN (Table 4), and this value was not significantly different from the inhibition observed with γ -IFN only ($P > 0.2$).

Effect of TGF- β on collagen synthesis by fibroblast subtypes

This experiment was performed on two strains of a fibroblast culture which were derived from the same

gingival explant under two growth conditions. One strain was obtained in medium containing 10 % fresh human serum (FS cells) and the other in medium with 10 % heat-inactivated plasma-derived serum (PS cells) (Bordin & Page, 1988). These cell strains were selected because they exhibit heritable differences in growth rate and cell-surface characteristics (Bordin & Page, 1988). The cells were exposed to TGF- β and collagen production and mRNA levels were measured. Fig. 2 shows that the PS

Fig. 2. Effect of TGF- β on collagen production and collagen mRNA contents of FS and PS substrains of human fibroblasts

The fibroblasts were exposed to 40 pM-TGF- β , after which collagen production (a) and mRNA (b) were measured as described in the Experimental section. In (a) means $\pm$ S.D. of triplicates are plotted. $\square$, No TGF; $\blacksquare$, + TGF.

cells produced approximately half as much collagen as did FS cells. TGF- β stimulated collagen production in both cell strains. The stimulation was greater in the PS cells (4.7-fold versus 2.4-fold in the FS cells) and as a result, both cells produced similar amounts after TGF- β treatment (Fig. 2a). Collagen mRNA data were also consistent with these results (Fig. 2b).

DISCUSSION

Our data on stimulation of collagen synthesis (measured in terms of collagen production and mRNA) by TGF- β and serum, and inhibition by γ -IFN, agree in general with those of others (Manner, 1971; Narayanan & Page, 1977, 1987; Rosenbloom *et al.*, 1984; Wrana *et al.*, 1986; Ignatz & Massagué, 1986; Czaja *et al.*, 1987). The degree of stimulation by TGF- β is also comparable with that observed by others in human fibroblasts (Wrana *et al.*, 1986; Raghov *et al.*, 1987). However, Wrana *et al.* (1986) reported only marginal stimulation in confluent cultures, whereas we observed that both confluent and subconfluent cultures responded equally well to TGF- β (2.0 ± 0.3 -fold, $n = 3$, and 2.0 ± 0.3 -fold, $n = 4$, respectively). Although serum and TGF- β appear to stimulate synthesis of all proteins, γ -IFN inhibits collagen synthesis without significantly affecting total incorporation and poly(A)⁺ mRNA (Rosenbloom *et al.*, 1984). It is possible that the differences in production observed in our experiments arose because of changes in specific radioactivities of the amino acids in the precursor pool. In the treated cells differences in specific radioactivity of precursor amino acids can arise owing to (a) variations in the intracellular concentration of unlabelled amino acids that dilutes the radioactive precursor at the time of its addition, and (b) changes in the rates of cellular protein degradation, a process which continuously dilutes the radioactivity of intracellular pool throughout the period of measurement. The former possibility appears unlikely, because: (1) cells were preincubated in serum-free medium before labelling, to minimize differences in pool size; (2) the same results were obtained by increasing the concentration of the labels 4-fold, and thus their specific radioactivity (results not shown); (3) the effect of the three components persists for up to 24 h after removal from the medium; and (4) protein production is associated with a parallel change in mRNA amounts. In similar experiments Wrana *et al.* (1986) found that TGF- β exposure does not affect amino acid transport signifi-

cantly. To what extent changes in the rate of cellular protein breakdown contribute to differences in specific radioactivity of amino acids is not clear. Overall *et al.* (1989) have observed that TGF- β suppresses the expression of procollagenase while stimulating the tissue-inhibitor of metalloproteinases and plasminogen activator-inhibitor. Serum, however, does not appear to affect collagen degradation significantly (Narayanan & Page, 1987). Even though the fact that collagen production and mRNA amounts are affected in parallel indicates that contribution of protein breakdown may be minimal, additional experiments are necessary to determine its precise role.

Collagen production and mRNA amounts are affected in parallel by TGF- β , serum and γ -IFN, indicating that their action is mediated through mRNA amounts and not through post-translational mechanisms. However, from our experiments it is not possible to determine whether changes in mRNA amounts arise from an effect on the rate of gene transcription or post-transcriptional mRNA-processing reactions. The magnitude of stimulation in synthesis induced by TGF- β reported here and by others for human fibroblasts (Wrana *et al.*, 1986; Raghov *et al.*, 1987) is far less than the 10–20-fold observed by Penttinen *et al.* (1988). Whether or not this is due to measurements being done after the time of peak effect, or to species differences (mouse versus human) is not known.

Even though fetal bovine serum contains 0.1–5.0 ng of TGF- β /ml the TGF- β does not appear to contribute to the serum effect, because it is present in serum as an inactive complex with α_2 -macroglobulin (O'Connor-McCourt & Wakefield, 1987), and because serum stimulation occurs even in the presence of optimal TGF- β concentrations. Our data also indicate that collagen production and DNA synthesis are not coupled together; thus, although serum enhances both collagen synthesis and fibroblast proliferation, TGF- β stimulates only the former. This conclusion is also supported by the fact that serum stimulation of collagen synthesis occurs in confluent cultures in which < 5% of the cells respond mitogenically to serum (Narayanan & Page, 1987).

Our experiments were designed mainly to obtain information about how collagen synthesis by fibroblasts is affected when the cell is presented with more than one inflammatory mediator. The magnitude of stimulation of collagen synthesis by TGF- β is not affected by serum [collagen production increased 3.0 ± 0.4 and 3.3 ± 1.0 -fold ($n = 3$) in the presence of 20% and 100% serum

respectively]. In the presence of both components stimulation is roughly equal to the total obtained with each component alone (Table 1). Similarly, stimulation by TGF- β was the same in cultures containing and previously treated with γ -IFN (Tables 2 and 3). γ -IFN inhibition was also the same in control and TGF- β -treated cultures (Tables 2 and 4). These data indicate that TGF- β , serum and γ -IFN are effective independently of each other. The rate of gene transcription is regulated by synergistic interactions of several factors such as *cis*-acting DNA sequences (promoters and enhancers), and *trans*-acting protein factors. The enhancers appear to contain different elements for binding different regulatory substances, and the *trans*-acting factors interact with the DNA elements through mechanisms that involve co-operative binding or other protein-protein interactions (Khoury & Gruss, 1983; Voss *et al.*, 1986; Sassone-Corsi & Borrelli, 1986). The fact that TGF- β , serum and γ -IFN each can affect collagen synthesis independently of each other indicates that these components perhaps exert their action by different mechanisms, or by interacting with different regulatory sequences on the collagen gene. Similar differential regulation has been observed for the proto-oncogene *c-fos* by growth factors (Verma & Sassone-Corsi, 1987). In collagen genes, enhancer DNA sequences are located in the first intron, and 274 bp- and 1106 bp-long sequences have been identified in human $\alpha 1$ [I] and mouse $\alpha 2$ [I] collagen genes respectively (Rossouw *et al.*, 1987; Bornstein *et al.*, 1987; Rossi & de Crombrughe, 1987). However, TGF- β and serum effects are not specific, so therefore their action is likely to involve common mechanisms which affect the expression of collagen as well as other genes. Persistence of their effect even after 24 h after their removal indicates that their mechanism of action may involve synthesis of molecules, perhaps nuclear proteins, that serve as mediators. This possibility is supported by the observation that cycloheximide, an inhibitor of protein synthesis, abrogated the TGF- β stimulation of collagen mRNA production (Penttinen *et al.*, 1988).

Our results indicate that the combined stimulation of collagen synthesis by TGF- β , serum and other substances can be beneficial during wound healing and tissue repair when the matrix must be reconstituted rapidly. On the other hand, the ability of γ -IFN (and other molecules) to inhibit collagen synthesis, even in the presence of TGF- β , will help to control excess production when tissue repair is complete or near completion (Ross, 1968, 1986; Dvorak, 1986). Our data also indicate that, even though the synthetic activities of fibroblast subpopulations are affected similarly by mediators such as TGF- β , once these substances are not available, the types of cell subpopulation remaining will determine whether the tissue becomes normal or pathologically altered. For example, the presence of cell populations with abnormally high steady-state amounts of collagen synthesis have been reported to be a contributory factor for fibrosis (LeRoy, 1972; Hassell *et al.*, 1976; Narayanan *et al.*, 1988).

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