# Inhibitory Effect of Gamma Interferon on Cultured Human Keratinocyte Thrombospondin Production, Distribution, and Biologic Activities

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Rapidly proliferating keratinocytes (KCs) maintained in low calcium, serum-free medium produce and utilize thrombospondin (TSP) as an attachment and spreading factor. To begin to understand the modulation of KC TSP metabolism, gamma interferon (IFN-$\gamma$), a product of activated T lymphocytes, was added to KC cultures. IFN-$\gamma$ was chosen because activated T cells appear at sites of cutaneous injury. Two additional cytokines including tumor necrosis factor (TNF) and IFN-$\beta$ were also examined. IFN-$\gamma$ (600 U/ml), but not TNF (500 U/ml) or IFN-$\beta$ ($10^3$ U/ml), as single agents decreased KC TSP biosynthesis, secretion, and utilization as an attachment factor. IFN-$\gamma$ alone did not detectably decrease TSP mRNA levels suggesting a post-transcriptional effect in KCs. However, the combination of IFN-$\gamma$ (600 U/ml) and TNF (500 U/ml) inhibited TSP mRNA production. These results demonstrate the modulation of KC TSP metabolism and biologic activity. *J Invest Dermatol 91:213–218, 1988*

I n normal skin, thrombospondin (TSP) is located in the basement membrane at the dermo-epidermal junction [1]. Recently, it has been shown that cultured human keratinocytes (KCs) synthesize TSP [2]. The synthesized material is deposited in the extracellular matrix (ECM) and serves as an adhesion factor for KCs [3]. Although cell-matrix interactions are thought to be critical to normal skin development as well as to reepithelialization following injury, little is known about how these interactions are regulated [4–7]. In order to begin probing the regulation of KC-matrix interactions, we examined the possible influence of a product of activated T lymphocytes (i.e., gamma interferon [IFN-$\gamma$]) on the production of TSP by KCs and on the response of KCs to TSP. IFN-$\gamma$ was examined because activated T lymphocytes appear at sites of skin injury [8] and because IFN-$\gamma$ induces detachment and shedding of cultured KCs from the substratum [9].

Abbreviations:
BSA: Bovine Serum Albumin
ECM: Extracellular matrix
ELISA: Enzyme-linked Immunosorbent Assay
IFN: Interferon
KC: Keratinocyte
MEM: Minimal Essential Medium of Eagle
MOPS: 3-(N-morpholino) propanesulfonic acid
SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TCA: Trichloroacetic acid
TNF: Tumor necrosis factor-alpha
TSP: Thrombospondin
U: Units

In the present study, we report that IFN-$\gamma$ prominently decreases TSP production by human KCs. In addition, it reduces KC attachment and spreading. Two other inflammatory mediators, tumor necrosis factor (TNF) and beta interferon (IFN-$\beta$), when used as single agents, do not have these effects. Not only do these studies add yet another effect of IFN-$\gamma$ on KCs [9–13], but they also suggest that TSP should be added to the list of potentially important molecules such as fibronectin [5–7] involved in cutaneous wound healing [14].

## MATERIALS AND METHODS

**Human Keratinocyte Culture** Primary KC cultures were initiated from normal skin obtained from adults after face-lift surgery using the method of Liu and Karasek [15]. Small, round, viable KCs ($1.7–2.0 \times 10^6$) were seeded onto 35 mm collagen-coated culture dishes (Lux, Flow Laboratories, McLean, VA) as previously described [9]. Culture medium consisted of modified MCDB 153 medium (KGM; Clonetics; San Diego, CA). After 1–3 d, the KCs were passaged and grown on plastic culture dishes without collagen in KGM. Under these conditions, the KCs remained in a rapidly growing condition [16]. The KCs were grown in a humidified incubator at 37°C with 7% $CO_2$/93% air, and cells at passage number 2–4 were routinely used in these experiments.

**Interferons and TNF** Recombinant IFN-$\gamma$ was obtained from Dr. M. Shepard (Genentech, Inc., San Francisco, CA) and had a specific activity of $3 \times 10^7$ units/mg (U/mg). The activity of the IFN-$\gamma$ was monitored using a sensitive radioimmunoassay procedure (Centocor, Melvern, PA). IFN-$\beta$ was obtained from Dr. T. Basham (Stanford University, CA) and had specific activity of $4.5 \times 10^7$ U/mg. The activity of the IFN-$\gamma$ was monitored using a viral plaque inhibition assay. IFN-$\beta$ was included in these studies because KCs produce IFN-$\beta$ after herpes simplex virus infection, where epidermal damage and reepithelialization are accompanied by an activated mononuclear cell infiltrate [17,18]. Recombinant tumor necrosis factor-$\alpha$, TNF, was obtained from Dr. M. Shepard (Genentech, Inc.) and had a specific activity of $5 \times 10^7$ U/mg. The

activity of the TNF was monitored using the cytopathic effect on sensitive target cell lines as performed by Dr. S. Kunkel (Department of Pathology, University of Michigan).

**Thrombospondin and anti-thrombospondin** TSP was purified from the releasate of thrombin-stimulated platelets by a combination of heparin-Sepharose and gel filtration chromatography as previously described [19]. Rabbit polycolonal antibodies to TSP were used in these experiments. The production and characterization of the polyclonal antibodies have been described previously [20,21]. Prior to use, the antibodies were affinity purified on a protein A-sepharose column.

**Enzyme-Linked Immunosorbent Assay (ELISA)** ELISAs were performed to determine the amount of immunoreactive TSP secreted into the culture medium by KCs under various conditions. After various intervals of treatment with IFNs or TNF, 2-h culture fluids were harvested, clarified by low-speed centrifugation, and 200 $\mu$l aliquots were added to wells of a 96-well plate (Falcon Plastics, Oxnard, CA) from lots that had been prescreened for acceptability in ELISAs. Purified human platelet TSP ($5 \times 10^{-1}$ – $5 \times 10^{-4}$ $\mu$g/well) was used to generate a standard curve and the ELISA was performed as previously described [22].

**Biosynthetic Labeling** To determine the amount of TSP produced by the KCs under various conditions, KCs were grown in 100 mm (diameter) plastic culture dishes to an approximate density of $3 \times 10^6$ KCs/dish. The KCs were washed and incubated for 30 min in methionine-free, serum-free minimal essential medium of Eagle with Earle's salts (MEM) (Flow Laboratories, McLean, VA), followed by a 4-h incubation in the same medium supplemented with 100 $\mu$Ci per dish of $^{35}$S-methionine ($1 \times 10^3 \mu$Ci/$\mu$mole, New England Nuclear, Boston, MA). After the 4-h treatment, the KCs were processed as previously described [23]. Briefly, KC lysates were frozen at $-80°$C, thawed, and clarified. Immunoreactive TSP was precipitated from the lysates by sequential treatment with polyclonal antibodies to TSP (or normal rabbit globulin) and protein A-Sepharose. The precipitated material was separated by electrophoresis on a 3%–10% gradient polyacrylamide gel under reducing conditions using the Laemmli system [24]. Radioactive bands were visualized by autoradiography using En$^3$Hance (New England Nuclear, Boston, MA) and developing the dried gels on x-ray film (Kodak XAR-2, Rochester, NY) for 2 d.

**TSP mRNA and Northern Blot Analysis** Total RNA for Northern blot analysis was prepared from the KCs using the method of Chirgwin et al [25]. Typically, yields from one 10-cm-diameter dish were between 50–100 $\mu$g of total RNA.

For Northern analysis 10-$\mu$g samples of total RNA were electrophoresed through an agarose gel formed in 2.2 M formaldehyde/20 mM 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (pH 7.25) 0.1 mM Na acetate, and electroblotted onto nitrocellulose in 10 mM Tris (pH 7.8) 5 mM Na acetate and 0.5 mM EDTA as described by Thomas [26]. The filters were baked in vacuo at 80°C for 2 h and pre-hybridized in 40% formamide/0.6 M NaCl/60 mM Na citrate/5X Denhardt's solution (1X Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/herring sperm DNA (100 mg/ml/0.1% NaDod SO$_4$/0.2% Na pyrophosphate/poly(A) (1 $\mu$g/ml) at 42°C for at least 3 h. Filters were washed in two changes of 0.3 M NaCl/30 mM Na citrate/0.1% NaDod SO$_4$ at 25°C for 10 min and two changes of 75 mM NaCl/75 mM Na citrate/0.1% NaDod SO$_4$ at 50°C for 1 h each. The filters were exposed to Kodak XAR-5 film with Dupont Cronix intensifying screens at $-70°$C. A cDNA probe consisting of the heparin binding domain of the TSP molecule [27] was labeled with [$^{32}$P]dATP to a specific activity of $2-5 \times 10^6$ dpm/ng by oligo-labeling [28] and used to identify TSP specific mRNA. The transfer of equivalent amounts of total RNA was ensured by examining the nitrocellulose transfer under UV light which allowed visualization of the 28s and 18s ribosomal bands.

**Indirect Immunofluorescence Staining** The KCs were examined for anti-TSP binding under various conditions by indirect im-

munofluorescence staining as previously described [23]. Briefly, KCs grown on glass coverslips were washed in cold PBS and stained at 4°C using a 1 : 20 dilution of rabbit anti-TSP or normal rabbit globulin (45 min) followed by a 1 : 50 dilution of fluorescein-conjugated swine anti-rabbit serum (45 min, Accurate Scientific and Chemical Company, Westburg, NY). The slides were examined and photographed using an Olympus EH-2 microscope.

**Adhesion Assay** KC substrate attachment and spreading were measured as previously described [23,29]. Briefly, various amounts of TSP were incubated for 2 h in 24-well culture plates (Costar, Cambridge, MA) using KGM as buffer. Next, the wells were washed and incubated with KGM containing 1% bovine serum albumin (BSA). KCs to be used in the adhesion assay were harvested following brief trypsinization (0.03% Trypsin, 0.01% EDTA −5 min) and added to untreated wells or to TSP-coated wells in KGM containing 1% BSA. At various time intervals, the non-attached KCs were removed from the wells and counted with an electronic particle counter. The wells were then washed twice and fixed by adding 2% glutaraldehyde. The percentage of spread KCs was determined using a phase contrast microscope with a calibrated grid.

## RESULTS

**Effects of IFN-$\gamma$ on TSP Biosynthesis by KCs** In the first series of experiments, biosynthetic labeling and immunoprecipitation were used to identify effects of IFN-$\gamma$ on TSP biosynthesis. KC cultures were treated with 600 U/ml of IFN-$\gamma$ for 48 h. Other cultures received both 600 U/ml of IFN-$\gamma$ and 500 U/ml of TNF or 500 U/ml of TNF alone for the same period. Control KC cultures were maintained under the same conditions but not exposed to IFN-$\gamma$ or TNF. After the 48-h preincubation period, all cultures were pulsed for 4 h with $^{35}$S-methionine and examined for TSP biosynthesis as described in the "Materials and Methods" section. Figure 1 shows an autoradiogram from such an experiment. It can be seen from this figure that when radiolabeled cell extracts were immunoprecipitated with rabbit polyclonal antibodies to TSP and examined by SDS-PAGE under reducing conditions, a single labeled band ($M_r = 180$ kD) was observed. There were no apparent qualitative differences in the immunoprecipitated material from any of the groups. However, there appeared to be significant differences in the amount of material precipitated. Specifically, the KCs treated with IFN-$\gamma$ produced less TSP than the control KCs. Simultaneous treatment with IFN-$\gamma$ and TNF appeared to enhance the inhibition of TSP synthesis but TNF treatment by itself had very little effect.

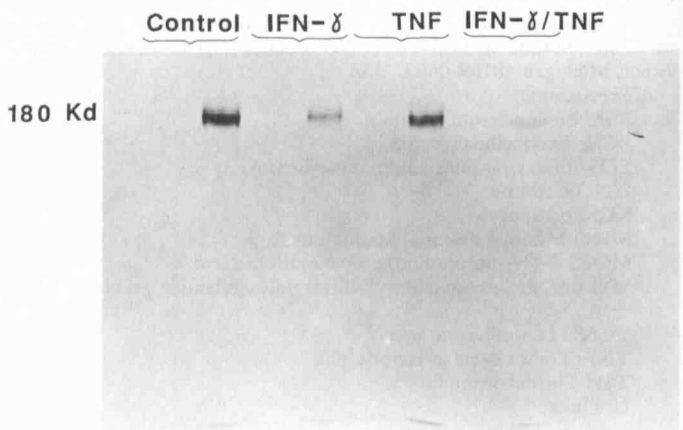In order to quantitate the differences in TSP production that were



**Figure 1.** Influence of IFN-$\gamma$ and TNF on TSP biosynthesis. Untreated KCs (control) and KCs treated with 600 U/ml of IFN-$\gamma$, 500 U/ml of TNF, or 600 U/ml of IFN-$\gamma$ and 500 U/ml of TNF were analyzed for TSP biosynthesis as described in "Materials and Methods". To the left of each immunoprecipitate is the same extract precipitated with normal rabbit serum.

evident from the autoradiogram, samples of immunoprecipitated material from each group were counted in a scintillation counter for total radioactivity and compared to the amount of trichloroacetic acid (TCA) (10% final concentration) precipitable radioactivity in the same sample (Table I). While the overall amount of TCA-precipitable material was similar for all four groups (suggesting that total protein synthesis was not significantly affected by IFN-γ and/or TNF), the amount of radioactivity precipitated with anti-TSP was reduced in the group treated with either IFN-γ alone or a combination of IFN-γ/TNF. TNF treatment by itself reduced the amount of anti-TSP precipitable radioactivity only slightly. In additional studies (not shown) we observed no differences between control cells and cells treated with 600 U/ml of IFN-γ in TSP production when the cells were pulsed with $^{35}$S-methionine immediately upon addition of IFN-γ. However, pretreatment of KCs with IFN-γ for as little as 2 h led to a reduction in TSP biosynthesis of approximately 30%.

**Effects of IFN-γ on TSP mRNA Production**  Figure 2 reveals that 48-h exposure to either IFN-γ (600 U/ml) *(lane 3)* or TNF (500 U/ml) *(lane 4)* alone produced only slight changes in TSP mRNA expression when compared to control KC cultures *(lane 2)*. The TSP mRNA detected in the KC cultures has an identical size to that observed for squamous carcinoma cells *(lane 1)* as previously reported [27]. When IFN-γ and TNF were combined, the TSP mRNA was dramatically reduced. To determine if IFN-γ (600 U/ml) or TNF (500 U/ml) when used as single agents may have altered TSP mRNA production at earlier time points, we also examined Northern Blots at 4 and 24 h but found no difference in levels of TSP mRNA when compared to control KC cultures (Not Shown).

**Effects of IFN-γ on TSP Secretion**  ELISAs were used to estimate the amount of TSP secreted into the culture medium by KCs maintained under control conditions or treated with IFN-γ. As shown in Table II, KCs treated for 24 or 48 h with IFN-γ secreted significantly less TSP into the culture fluid than control KCs. Treatment of KCs for shorter periods of time (2–6 h) did not result in decreased TSP secretion into the culture medium. Table II also shows that the simultaneous treatment of KCs with IFN-γ and TNF further reduced TSP secretion. In contrast to the effects of INF-γ, treatment of KCs with IFN-β did not inhibit TSP secretion. Dose-response studies were also carried out using IFN-γ. At 1 U/ml there was no detectable inhibition of TSP secretion. TSP secretion was inhibited at concentrations as low as 5 U/ml and inhibition was dose-responsive over the range of 5–300 U/ml. In contrast, no inhibition was observed when TNF was used alone at concentrations between 5–3000 U/ml (not shown).

**Surface Expression of TSP by Untreated and IFN-γ Treated KCs**  KCs were grown for 48 h on glass coverslips under control conditions or in the presence of 600 U/ml of IFN-γ. Following this, they were stained in the viable state for immunofluorescence as described in "Materials and Methods" and examined by fluores-
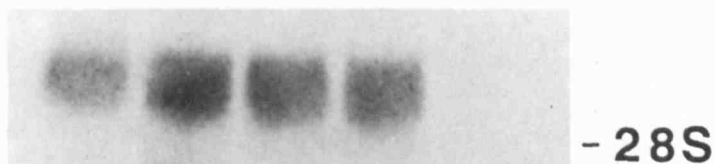


**Figure 2.** Influence of IFN-γ and TNF on TSP mRNA levels: 10 μg of total RNA was prepared from Human squamous carcinoma cells (Sq), untreated KCs (KC/c), IFN-γ treated KCs (IFN-γ), TNF treated KCs (TNF), IFN-γ and TNF treated KCs (IFN-γ/TNF), and analysed by Northern blotting as described in "Materials and Methods".

cence microscopy (Fig 3). While the control cells stained brightly with anti-TSP, there was significantly less staining of the IFN-γ-treated cells. Interestingly, the coverslips from both the control cells and the IFN-γ treated cells showed a hazy intercellular fluorescence with anti-TSP. This pattern of fluorescence was not observed when normal rabbit globulin was substituted for the anti-TSP and most likely represents TSP absorbed onto the surface of the coverslip.

**Effect of IFN-γ on KC adhesion**  KCs were examined for ability to attach and spread on plastic culture dishes in the presence of BSA and for ability to attach and spread on dishes coated with 25 μg of TSP. Untreated KCs maintained in KGM attached and spread rapidly on TSP-coated dishes (Fig 4). A significant number of cells were attached and spread by 1 h after plating, and the number increased through 5 h of incubation. Consistent with our previous observations [3] dose-response studies indicated that amounts of TSP as low as 0.5 μg per dish were sufficient to elicit a response, and pretreatment of the TSP with polyclonal antibodies to TSP inhibited the response (not shown). Untreated KCs also attached and spread on plastic culture dishes in the presence of BSA, but the rate of attachment and spreading, as well as the overall percentage of cells which responded, was lower than on dishes coated with TSP (Fig 4). In contrast to these effects with untreated KCs, pre-treatment of the KCs for 48 h with 600 U/ml of IFN-γ dramatically reduced attachment and spreading on both TSP-coated dishes and plastic dishes (Fig 4). Interestingly, the treated cells failed completely to attach and spread on plastic dishes in the presence of BSA but did begin to attach and spread on TSP-coated dishes. A peak response was observed at approximately 3 h, but the cells detached subsequent to this. The explanation for this is not fully understood, but may involve multiple binding sites for TSP, only one of which is directly affected by IFN-γ.

**Table I.**  Estimation of Immunoreactive TSP Biosynthesis in Cell Extracts of Control and Treated KCs*

| Group | TCA- precipitated cpm | Anti-TSP precipitated cpm | % TSP |
|---|---|---|---|
| Control | $4.66 \pm 2.18 \times 10^6$ | $2.7 \pm 0.1 \times 10^4$ | 0.58 |
| IFN-γ | $6.10 \pm 2.50 \times 10^6$ | $8.5 \pm 0.6 \times 10^3$ | 0.14 |
| TNF | $4.84 \pm 1.94 \times 10^6$ | $1.9 \pm 0.1 \times 10^4$ | 0.40 |
| IFN-γ/TNF | $5.50 \pm 0.83 \times 10^6$ | $3.3 \pm 1.6 \times 10^3$ | 0.06 |

* $3 \times 10^6$ cells in 100 mm dishes were pulsed for 4 h with $^{35}$S-methionine. Cell lysates were prepared from the labeled cells and divided into two parts. One part was precipitated with anti-TSP and the other part was precipitated with normal rabbit globulin. A small sample from each part was also precipitated with 10% TCA. The amount of radioactivity precipitated with normal rabbit globulin was subtracted from the amount precipitated with anti-TSP. The remaining value was compared with the amount precipitated with 10% TCA and expressed as a percentage of the total. Values shown are averages ± standard deviations based on three separate experiments.

**Table II.**  Immunoreactive TSP in the Culture Fluid of Control and Treated KCs*

| Group | TSP (ng/ml/$10^6$ KCs) | P Value |
|---|---|---|
| Control | $500 \pm 76$ | |
| IFN-γ (2 h) | $470 \pm 30$ | ns |
| IFN-γ (6 h) | $535 \pm 40$ | ns |
| IFN-γ (24 h) | $215 \pm 35$ | p < 0.05 |
| IFN-γ (48 h) | $150 \pm 27$ | p < 0.01 |
| TNF (48 h) | $310 \pm 15$ | p < 0.05 |
| IFN-γ/TNF (48 h) | $50 \pm 12$ | p < 0.001 |
| IFN-β (48 h) | $510 \pm 105$ | ns |

* KCs were grown in a 24-well culture dish and treated for the designated amount of time with 600 U/ml of IFN-γ, 500 U/ml of TNF, or 1000 U/ml of IFN-β. Two-hour culture fluids were then obtained and analyzed for immunoreactive TSP by ELISA. Values shown are averages ± standard deviations based on six separate experiments. Statistical significance levels of each value, compared to the untreated central value, was determined using Students' t-test. The values shown are averages ± standard deviations based on six separate experiments. ns: not significant at 0.05 level.
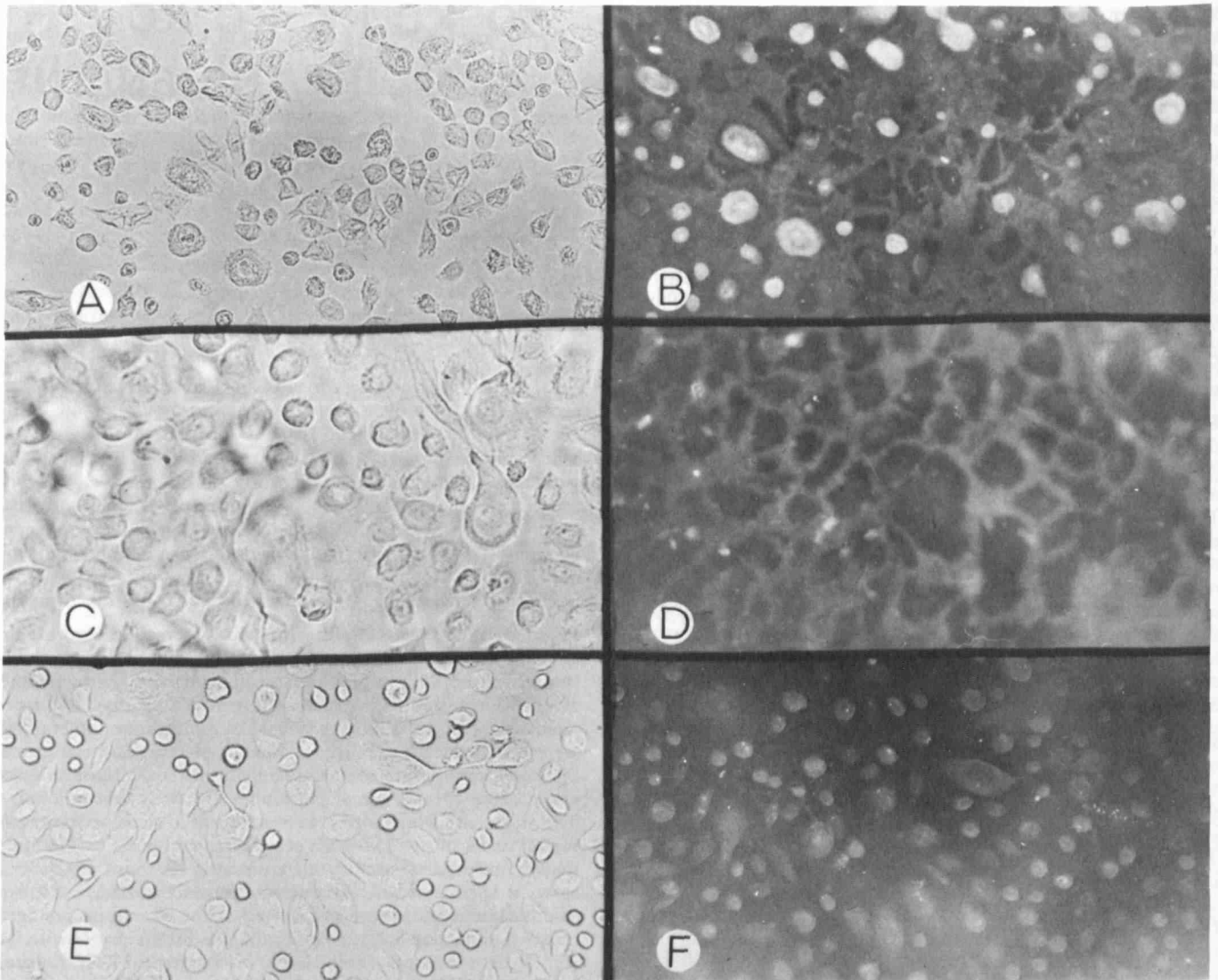
**Figure 3.** Indirect Immunofluorescence Microscopy of KC TSP distribution. In control, untreated cultures, numerous KCs stain brightly positive for TSP (*B*). After IFN-γ treatment (600 U/ml, 48 h), there is no KC staining for TSP, but the intercellular areas contain a hazy positive fluorescence (*D*). *F* depicts untreated cultures stained using non-immune control rabbit serum. *A, C* and *E* portray the phase contrast microscopy of identical right-hand-side view of immunofluorescence staining (100×).
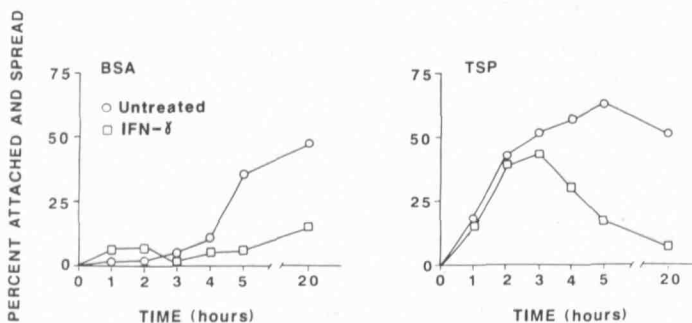


**Figure 4.** Effects of IFN-γ on KC adhesion. Untreated KCs and KCs treated with 600 U/ml of IFN-γ for 48 h were examined for attachment and spreading on plastic culture dishes in the presence of BSA and on dishes coated with 25 μg of TSP. The values shown represent the average percentage of cells which were attached and spread at each time point, based on duplicate samples in a single experiment. The individual values were within 15% of the averages. The experiment was repeated three times with similar results.

## DISCUSSION

There has recently been much interest in the role of the ECM in normal skin development and in cutaneous wound healing. Much of the recent interest, particularly in relation to wound healing, has centered on fibronectin [5–7,30,31]. We have observed recently, however, that KCs attach and spread to a greater extent on TSP than on fibronectin in vitro [3] and a variety of evidence suggests that TSP may play an important role in mediating KC attachment to the underlying basement membrane in vivo. First, it is prominently expressed at the dermal-epidermal junction in vivo where basal keratinocytes are adherent to the basement membrane [1]. Second, it is produced in significant quantities by undifferentiated KCs but not by KCs which have been induced to differentiate in the presence of high $Ca^{2+}$ [3]. Finally, basal adhesion of KCs (e.g., adhesion to the substrate in the absence of exogenous adhesion factors) can be inhibited with antibodies to TSP [3]. Based on these data, we suggest that TSP is synthesized by rapidly proliferating, undifferentiated KCs and secreted into the culture medium. The secreted TSP then binds to the surface of the cell that secreted it or to adjacent cells to induce cell-substrate attachment by an autocrine or paracrine mechanism. In support of this is the fact that the amount of TSP secreted into the

culture medium by untreated KCs (approximately 0.5 μg per ml in 4–6 h) is sufficient to account for the adhesion to the plastic culture dishes in the presence of BSA but in the absence of exogenous adhesion factors.

A major goal of the present study was to examine the effects of IFN-γ on the production and distribution of TSP by human KCs and on the utilization of this moiety as an adhesion factor. Our studies showed that this product of activated T lymphocytes induces a rapid decrease in TSP production by KCs in vitro. Accompanying decreased TSP production is an inhibition of TSP secretion into the culture medium and a decreased expression of TSP on the cell surface. The ability of IFN-γ to decrease KC TSP production is not due to a net reduction in total protein synthesis. We previously reported that some other proteins are increased (such as HLA-DR) by the same type of IFN-γ treatment [10,32]. The lack of significant changes in amount of TSP mRNA after IFN-γ treatment alone suggests that post-transcriptional modification may be more important for KCs than the transcriptional control for TSP. Because TSP requires glycosylation prior to secretion and because interferons are known to inhibit glycosylation [33], this may be one specific cellular metabolic event influenced by IFN-γ. In contrast, combined treatment of KCs with IFN-γ and TNF dramatically inhibited TSP mRNA production. This synergistic effect may account for the near total inhibition of TSP biosynthesis and secretion. In addition to decreasing TSP biosynthesis and secretion, IFN-γ also inhibited TSP expression on the cell surface as indicated by indirect immunofluorescence. Decreased TSP expression on the cell surface may be related in part to decreased synthesis and secretion but may also be due to altered TSP binding. In previous studies, we observed a direct relationship between TSP biosynthesis and TSP binding capacity among human squamous carcinoma cell lines [34]. Furthermore, our recent studies have shown that IFN-γ treated KCs bind significantly less TSP than untreated KCs and the decrease in binding is due almost entirely to a decrease in the specific component of the total.

It has been shown previously that IFN-γ causes shedding of cultured KCs [9] and the present study shows that KC attachment to untreated culture dishes in the presence of BSA or to TSP-coated dishes was markedly affected by IFN-γ. Thus, it is possible that at least one way in which IFN-γ inhibits KC proliferation is by decreasing TSP production [9]. It should be noted that IFN-γ has been observed to inhibit collagen production both in vitro [35–37] and in vivo [38]. It has also been shown that IFN-γ and TNF act synergistically to produce a loss of stainable fibronectin in the ECM in vitro [39]. This suggests that these cytokines may affect a wide variety of ECM molecules. Interestingly, the regulation of collagen production by IFN-γ in fibroblasts and chondrocytes appears to occur at the level of transcription [35–37]. Our current data suggest that post-transcriptional events may be more important in the inhibition of TSP production by IFN-γ when used singly. In contrast, the synergistic effects of IFN-γ and TNF in decreasing TSP production appears to result from an effect on transcription. Other studies have also noted synergy between IFN-γ and TNF in modulating proliferation [40,41], ECM production [35], and in vivo inflammatory reactions [42,43]. Based on our current results, it is possible that other synergistic effects may be regulated at the transcriptional level.

In conclusion, the ability of IFN-γ to modulate TSP related metabolic events in rapidly proliferating KCs in vitro provides additional support for the notion that cytokines may participate in various inflammatory conditions of the skin involving activated mononuclear cells in vivo including would healing [8]. Integrating current results with information from several recent reports, it would appear that regulation of cellular events in wound healing may involve some cytokines that influence KC and fibroblast proliferation [44–46] as well as ECM synthesis [47,49].

## REFERENCES

1. Wight TN, Raugi GJ, Mumby SM, Bornstein P: Light microscopic immuolocation of thrombospondin in human tissues. J Histochem Cytochem 33:295–302, 1985

2. Wikner NE, Dixit VM, Frazier WA, Clark RAF: Human keratinocytes synthesize and secrete the extracellular matrix protein, thrombospondin. J Invest Dermatol 88:207–211, 1987

3. Varani J, Nickoloff BJ, Riser BL, Mitra RS, O'Rourke K, Dixit VM: Thrombospondin-induced adhesion of human keratinocytes. J Clin Invest (In press)

4. Woodley DT, O'Keefe EJ, Prunieras M: Cutaneous wound healing: A model for cell-matrix interactions. J Am Acad Dermatol 12:420–433, 1985

5. Toda K, Grinnell F: Activation of human keratinocyte fibronectin receptor function in relation to other ligand-receptor interaction. J Invest Dermatol 88:412–417, 1987

6. O'Keefe EJ, Woodley DT, Falk RJ, Gannon WR, Briggaman RA: Production of fibronectin by epithelium in a skin equivalent. J Invest Dermatol 88:634–639, 1987

7. Kubo M, Kam M, Isemura M, Yamane I, Tagami H: Effects of extracellular matrices on human keratinocyte adhesion and growth and on its secretion and deposition of fibronectin in culture. J Invest Dermatol 88:594–601, 1987

8. Reusch M, Nickoloff BJ, Mansbridge JN, Morhenn VB: Immunophenotyping of skin cells during healing of a suction blister injury. J Invest Dermatol 84:292, 1985

9. Nickoloff BJ, Basham TY, Merigan TC, Morhenn VB: Antiproliferative effects of recombinant alpha and gamma interferons on cultured human keratinocytes. Lab Invest 51:697–701, 1984

10. Basham TY, Nickoloff BJ, Merigan TC, Morhenn VB: Recombinant gamma interferon induces HLA-DR expression on cultured human keratinocytes. J Invest Dermatol 83:88–92, 1984

11. Morhenn VB, Nickoloff BJ, Mansbridge JN: Induction of the synthesis of triton soluble proteins in human keratinocytes by gamma interferon. J Invest Dermatol 85:27–29, 1985

12. Nickoloff BJ, Mahrle G, Morhenn VB: The ultrastructural effects of recombinant gamma interferon on cultured human keratinocytes. J Ultrast Res 10:17–21, 1986

13. Nickoloff BJ, Lewinsohn D, Butcher E, Krensky AM, Clayberger C: Recombinant gamma interferon increases binding of peripheral blood mononuclear leukocytes and a Leu 3+ T-lymphocyte cell clone to cultured keratinocytes and a malignant cutaneous squamous carcinoma cell line which is blocked by antibody against the LFA-1 molecule. J Invest Dermatol 90:17–22, 1988

14. Woodley DT: Importance of the dermal-epidermal junction and recent advances. Dermatologica 174:1–10, 1987

15. Liu SC, Karasek M: Isolation and growth of adult human epidermal keratinocytes in cell cultures. J Invest Dermatol 71:157–162, 1978

16. Boyce ST, Ham RG: Calcium regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum-free serial culture. J Invest Dermatol 81:335–405, 1983

17. Cunningham AL, Turner RR, Miller AC, Para MF, Merigan TC: Evolution of recurrent herpes simplex lesions. An Immunohistologic study. J Clin Invest 75:226–233, 1985

18. Torseth J, Nickoloff BJ, Basham TY, Merigan TC: Beta interferon produced by keratinocytes in human cutaneous herpes simplex infection. J Infect Dis 155:641–648, 1987

19. Dixit VM, Haverstick DM, O'Rourke KM, Hennessy SW, Grant GA, Santoro SA, Frazier WA: Monoclonal antibodies against human thrombospondin inhibit platelet aggregation. Proc Natl Acad Sci 82:3472–3476, 1985

20. Dixit VM, Haverstick DM, O'Rouke KM, Hennessy SW, Grant GA, Santoro SA, Frazier WA: Effects of anti-thrombospondin monoclonal antibodies on the agglutination of erythrocytes and fixed, activated platelets by purified thrombospondin. Biochem 24:4270–4278, 1985

21. Galvin NJ, Dixit VM, O'Rourke KM, Santoro SA, Grant, GA, Frazier WA: Mapping of epitopes for monoclonal antibodies against human platelet thrombospondin with electron microscopy and high sensitivity amino acid sequencing. J Cell Biol 101:1434–1441, 1985

22. Varani J, Lovett EJ, McCoy JP, Shibata S, Maddox DE, Goldstein IJ, Wicha M: Differential expression of a laminin-like substance by high and low metastatic tumor cells. Am J Path 111:27–34, 1983

23. Varani J, Dixit VM, Fligiel SEG, McKeever PE, Carey TE: Thrombospondin induced attachment and spreading of human squamous carcinoma cells. Exp Cell Res 167:376–390, 1986

24. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 268:685, 1970

25. Chirgwin JM, Przybyla AE, MacDonald RJ, Ruttler WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 24:5294–5299, 1979

26. Thomas P: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205, 1980

27. Dixit VM, Hennessy SW, Grant GA, Rotwein P, Frazier WA: Characterization of a cDNA encoding the heparin and collagen binding domains of human thrombospondin. Proc Natl Acad Sci USA 83:5449–5453, 1986

28. Feinberg A, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Analyt Biochem 132:6–13, 1983

29. Varani J, Carey TE, Fligiel SEG, McKeever PE, Dixit V: Tumor type-specific differences in cell substrate adhesion among human tumor cell lines. Int J Cancer 39:397–403, 1987

30. Kolata G: Understanding Wound Healing. J Invest Dermatol 88:351–352, 1987

31. Kolata G: Wound Healing and The Role of Fibronectin. J Invest Dermatol 88:527, 1987

32. Mansbridge J, Nickoloff BJ, Morhenn VB: Induction of new proteins by gamma interferon in cultured human keratinocytes. J Invest Dermatol 88:602–610, 1987

33. Maheshwari RK, Banerjee DK, Waechter CJ, Olden K, Friedman RM: Interferon treatment inhibits glycosylation of viral protein. Nature 287:454–455, 1980

34. Riser BL, Varani J, O'Rourke K, Dixit VM: Thrombospondin binding by human squamous carcinoma and melanoma cells: Relationship to Biological Activity. Exp Cell Res 174:319–329, 1988

35. Rosenbloom J, Feldman G, Freundlich B, Jiminez SA: Transcriptional control of human diploid fibroblast collagen synthesis by gamma interferon. Biochem Biophys Res Comm 123:365–372, 1984

36. Duncan MR, Berman B: Gamma Interferon is the lymphokine and β-interferon the monokine responsible for inhibition of fibroblastic collagen production and late but not early fibroblast proliferation. J Exp Med 162:516–527, 1985

37. Goldring MB, Sandell LJ, Stephenson ML, Krane SM: Immune interferon suppresses levels of procollagen mRNA and type II collagen synthesis in cultured human articular and costal cartilage. J Biol Chem 261:9049–9056, 1986

38. Granstein RD, Murphy GF, Margolis RJ, Byrne MH, Amento EP: Gamma interferon inhibits collagen synthesis in vivo in the mouse. J Clin Invest 79:1254–1258, 1987

39. Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am J Pathol 123:16–24, 1986

40. Lee SH, Aggarwal BB, Rinderknecht E, Assisi F, Chiu H: The synergistic antiproliferative effect of gamma interferon and human lymphotoxin. J Immunol 133:1083–1086, 1984

41. Fransen L, VanDer Heyden J, Ruysschaert F, Fiers W: Recombinant tumor necrosis factor: Its effort and its synergism with interferon-gamma on a variety of normal and transformed human cell lines. Ex J Cancer Clin Oncol 22:419–426, 1986

42. Shalaby MR, Aggrawal BB, Rinderknecht E, Sverdsky LP, Finkle BS, Palladino MA J.: Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factor. J Immunol 135:2069–2073, 1985

43. Talmadge JE, Bowersox O, Tribble H, Lee SH, Shepard HM, Liggitt D: Toxicity of tumor necrosis factor is synergistic with gamma interferon and can be reduced with cyclooxygenase inhibition. Am J Path 128:410–425, 1987

44. Ristow HJ: A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin-1 or a related protein. Proc Natl Acad Sci 84:1940–1944, 1987

45. Gilchrest BA, Sauder DN: Autocrine growth stimulation of human keratinocytes by epidermal derived thymocyte activating factor. Clin Res 32:585, 1984

46. Phan SH, McGarm BM, Loeffler KM, Kunkel Sl: Regulation of macrophage-derived fibroblast growth factor release by arachidonate metabolism. J Leuk Biol 42:106–113, 1987

47. Krane SM, Dayer JM, Simon LS, Byrne MS: Mononuclear cell conditioned medium containing mononuclear cell factor, homologous with interleukin-1, stimulates collagen and fibronectin synthesis by adherent synovial cells: Effects of prostaglandin $E_2$ and indomethacin. Collagen Res 5:99–117, 1985

48. Matsushima K, Bano M, Kidewell WR, Oppenheim JJ: Interleukin-1 increases collagen type IV production by murine mammary epithelial cells. J Immunol 134:904–909, 1985

49. Ignotz RA, Massague J: Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261:4337–4345, 1986

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The European Society for Dermatological Research and the International Society of Dermatopathology invite you to a clinically oriented symposium to be held in Liège, Belgium February 16–18, 1989. Guest lectures are planned and free communications are welcome. Deadline for submission of abstracts, 400 words maximum, is October 15, 1988. For further information please contact: Dr. G.E. Pierard, Service de Dermatopathologie, CHU du Sart Tilman, B-4000 Liege, Belgium