Transforming Growth Factor-β in Psoriasis

Pathogenesis and Therapy

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INTRODUCTION

Psoriasis is a common skin disease, affecting 1 to 2% of the U.S. population at a cost of some 1.5 billion dollars per year.[1] Psoriasis is characterized by marked increases in keratinocyte proliferation,[2] abnormal patterns of keratinocyte differentiation,[3] prominent alterations in dermal capillary vasculature,[4] and the presence of dermal and epidermal T cells, monocyte/macrophages, and polymorphonuclear leukocytes.[5,6] We have recently shown that expression of transforming growth factor-α (TGF-α) is markedly increased in psoriatic lesions.[7] This result, coupled with the fluctuating clinical course of psoriasis, suggests that the molecular pathogenesis of psoriasis may involve unstable or altered regulation of TGF-α and possibly other epidermally derived cytokines, such as interleukin-6 (IL-6).[8]

Transforming growth factor-β (TGF-β) acts synergistically with TGF-α to promote anchorage-independent growth of certain cell types.[9] However, it has potent antiproliferative effects upon a variety of epithelial cell types *in vitro*,[10] including keratinocytes.[11] In human keratinocytes, TGF-β causes a reversible inhibition of proliferation, predominantly in the G1 phase of the cell cycle.[12] On the basis of these results, it has been hypothesized that TGF-β functions as a negative growth regulator in normal skin.[11,12] Given the marked keratinocyte hyperproliferation characteristic of psoriasis, it is possible that psoriatic keratinocytes could be deficient in either the production of or responsiveness to TGF-β, resulting in a deficient state of growth regulation. We have tested this hypothesis in several different ways with respect to TGF-β1, the first member of the TGF-β gene family to be molecularly cloned and biochemically characterized. First, we have compared the expression of TGF-β1 mRNA in normal and psoriatic epidermis by Northern blotting. These results confirm those already reported using a slot blot procedure.[7] Second, we have compared the responsiveness of keratinocytes cultured from the skin of normal individuals and from psoriatic lesions to purified TGF-β1. The responses studied include TGF-β1-induced alterations in c-*myc* and plasminogen activator inhibitor-1 (PAI-1) mRNA levels as well as the proliferative response.

Finally, we have characterized the keratinocyte TGF-β1 receptor by Scatchard analysis and TGF-β1 crosslinking, and compared the receptors of normal and psoriatic keratinocytes. The results indicate that TGF-β1 is capable of acting directly on psoriatic as well as normal keratinocytes, and suggest that TGF-β could be an effective antipsoriatic agent.

MATERIALS AND METHODS

TGF-β1

Bone-derived TGF-β1 was purified from the noncollagenous, guanidine-HCl–soluble proteins of demineralized bovine bone as previously described.[13] For binding and receptor crosslinking studies, TGF-β1 was radiolabeled with 1 mCi of $Na^{125}I$ (15 mCi/μg, Amersham) as previously described.[14]

Keratinocyte Culture

Primary cultures of human keratinocytes were prepared by trypsinization of keratome or punch biopsies of skin removed from normal or psoriatic volunteers under lidocaine anesthesia. The procedures used have been described previously.[15-17] Cultures were propagated in Keratinocyte Growth Medium (KGM, Clonetics, Boulder, CO), a modification of MCDB 153 defined medium,[16] optimized for high density keratinocyte growth, and used in the second to fifth passage. For assays of keratinocyte proliferation, cells were seeded in KGM at 10^5 cells/60-mm dish (Falcon) and allowed to attach overnight. TGF-β1 (1 ng/ml) or diluent (0.1 M HCl, 1 mg/ml BSA) was then added and cells were trypsinized and counted at the indicated intervals using a hemacytometer.

RNA Isolation and Analysis

Keratinocytes (2–4 × 10^6 cells per 100-mm dish) were treated with TGF-β1 (1 ng/ml) or recombinant human γ-interferon (γ-IFN, Genentech, 100 U/ml) 24 h after feeding with KGM. After 3 h, cells were lysed with 4 M guanidinium isothiocyanate, 5 mM sodium citrate, pH 7.0, 100 mM beta-mercaptoethanol, 0.5% sodium sarcosinate, and RNA was isolated by overnight centrifugation at 100,000 × *g* over 5.7 M CsCl, 100 mM EDTA, pH 7.0.[17] For extraction of RNA from keratome biopsies of normal and psoriatic epidermis, biopsies were immediately frozen in liquid nitrogen after removal and stored at −70°C. The frozen biopsies were finely pulverized in liquid nitrogen prior to addition of guanidinium isothiocyanate buffer, then homogenized in a Polytron tissue grinder (Brinkmann). RNAs were quantitated by absorbance at 260 nm, and equal quantities of total RNA were size-fractionated on 1% formaldehyde-agarose gels.[18] RNAs were blotted onto derivatized nylon membranes (Zeta-probe, Bio-Rad) using 10 × SSC (1 × SSC = 0.15 M sodium chloride, 15 mM sodium citrate). Filters were hybridized against ^{32}P-labeled DNA probes prepared by random priming exactly as previously described.[18] cDNA plasmids containing TGF-β1 and PAI-1 inserts were generously provided by Drs. Rik Derynck of Genentech, Inc. and David Ginsburg of the University of Michigan, respectively.[19,20] *c-myc* and glyceraldehyde-3-phosphate de-

hydrogenase cDNA plasmids were obtained through the American Type Culture Collection. A lipocortin II cDNA probe was used for normalization of normal and psoriatic epidermal RNA loading and intactness.[33]

TGF-β1 Binding and Receptor Crosslinking Assays

Specific binding of radioiodinated TGF-β1 to cultured keratinocytes was determined as previously described.[21,22] Briefly, cell monolayers were rinsed several times in ice-cold binding buffer (Dulbecco's modified Eagle's medium containing 25 mM HEPES, pH 7.4, 0.1% BSA) followed by incubation with the indicated concentrations of ^{125}I-labeled TGF-β1 at 4°C for 3 to 4 h with gentle agitation. Cells were rinsed four times in ice-cold Hanks buffered salt solution containing 0.1% BSA, and solubilized in 1% Triton X-100, 10% glycerol, 0.01% BSA, 20 mM HEPES, pH 7.4. Bound and unbound TGF-β1 was then determined using a gamma counter. Nonspecific binding, determined by addition of 10 nM unlabeled TGF-β1, was subtracted from the total bound radioactivity.

^{125}I-labeled TGF-β1 was crosslinked to receptors on keratinocyte monolayers using disuccinimidyl suberate (DSS, Pierce) exactly as previously described.[22]

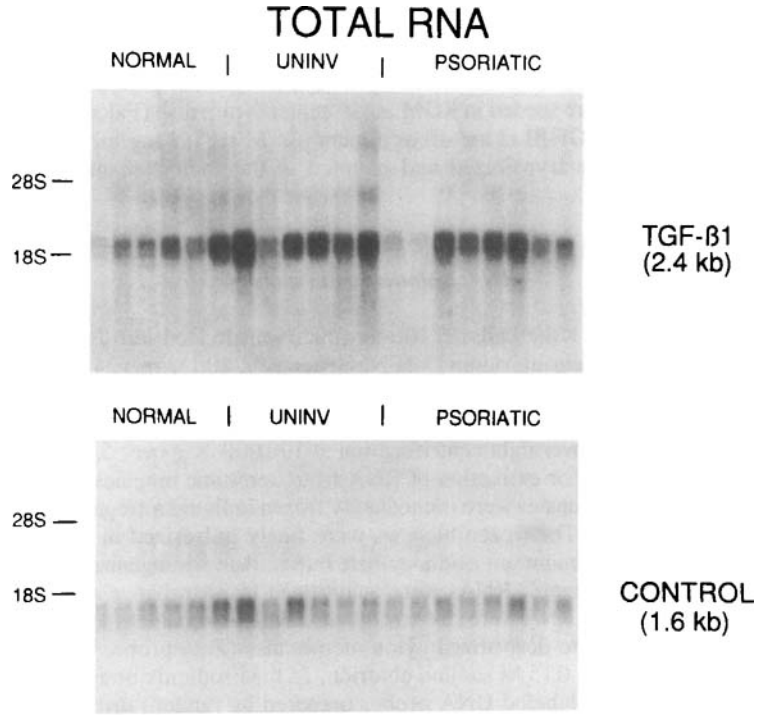


FIGURE 1. Northern blot analysis of TGF-β1 mRNA levels in normal, uninvolved, and lesional psoriatic epidermis. 18 S and 28 S ribosomal RNA mobilities are indicated to the left. (Upper panel) TGF-β1 probe. (Lower panel) Lipocortin II control probe.

RESULTS

In FIGURE 1, TGF-β1 steady-state mRNA levels in normal epidermis as well as in normal-appearing (uninvolved) and lesional psoriatic epidermis have been compared by RNA blot hybridization. A predominant band of approximately 2.4 kilobases (kb) is detected in all three sample types. Moreover, as reported previously using a slot-blotting technique,[7] the intensity of the 2.4 kb band is similar in all three groups, although some variation within each group is seen (upper panel). The lipocortin II control gene probe detects a 1.6 kb transcript at comparable intensities in all three groups (lower panel).

FIGURE 2 depicts the responses of several keratinocyte mRNA transcripts to treatment of cultures derived from normal epidermis or active psoriatic lesions with purified TGF-β1 (1 ng/ml; 40 pM). For a comparison, parallel dishes were treated with another cytokine known to inhibit keratinocyte proliferation, γ-IFN (100 U/ml). In each instance, TGF-β1 caused substantial decreases in c-*myc* mRNA 3 h after treatment (top panel). These results could not be accounted for by a nonspecific toxic effect, since re-hybridization of the same blots with the PAI-1 probe revealed markedly increased PAI-1 mRNA levels (second panel). Repeated hybridizations with the TGF-β1 probe

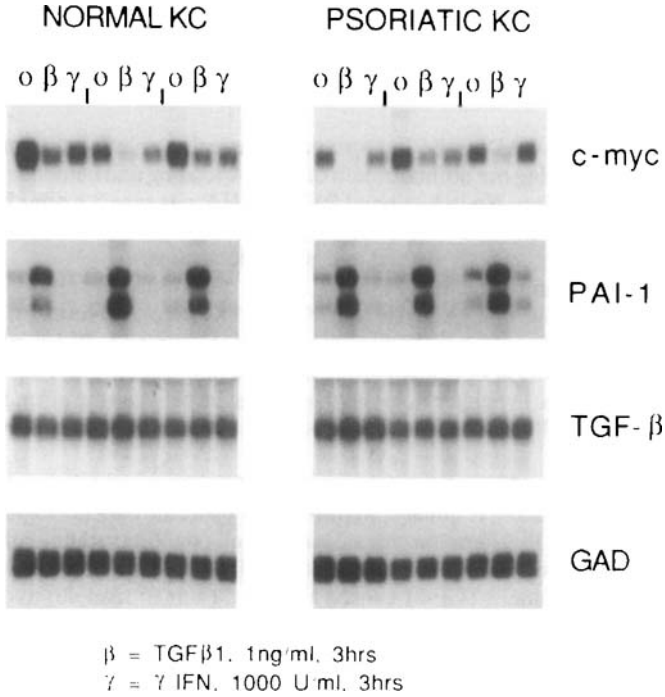


FIGURE 2. Keratinocyte mRNA responses to TGF-β1 and γ-IFN treatment. Only the relevant hybridizing bands of Northern blots loaded with 20 μg total cellular RNA are shown. Probes used for hybridization are indicated to the right. (o) No treatment. (β) TGF-β treatment. (γ) γ-IFN-treatment.

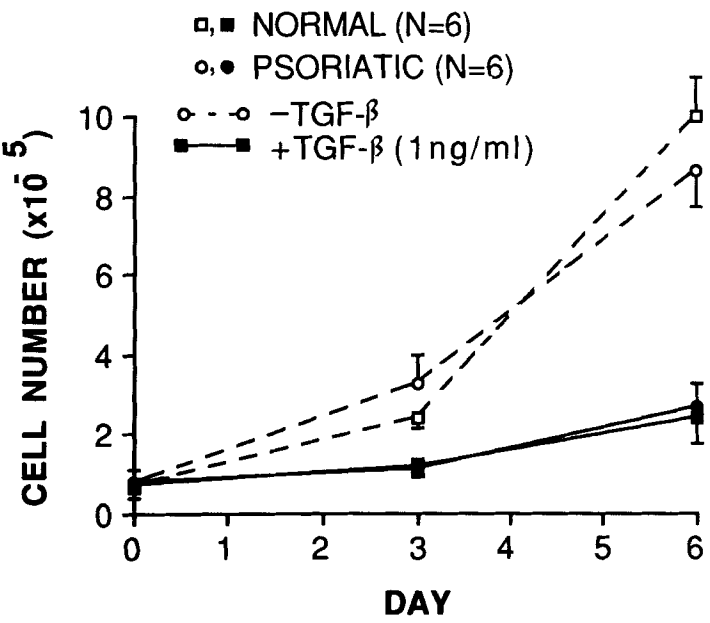


FIGURE 3. Proliferative responses of normal and psoriatic keratinocytes to TGF-β1 treatment.

(third panel) and the GAD probe (bottom panel) revealed no effects on mRNA transcript levels, confirming the normalization of mRNA loading and demonstrating a lack of TGF-β autoinduction. No induction of PAI-1 mRNA was seen 3 h after IFN-γ treatment, although there was a trend towards reduction of c-*myc* mRNA transcript levels in response to IFN-γ in both normal and psoriatic groups.

The autoradiograms depicted in FIGURE 2 were quantitated by laser densitometry. TGF-β1 reduced c-myc mRNA levels to 27.9 ± 3.9% of control levels in the normal group, and 21.4% of control in the psoriatic group. In contrast, PAI-1 mRNA levels were increased to 1,133% of control levels in the normal group, and 786% of normal in the psoriatic group. In neither case was the difference between the normal and psoriatic groups statistically significant, as judged by Student's *t* test using a two-tailed hypothesis ($p > 0.1$).

FIGURE 3 compares the proliferative responses of keratinocyte cultures derived from six normal individuals and lesional epidermis of six psoriatic patients to purified TGF-β1 (1 ng/ml, 40 pM). Proliferation was assayed by cell counting rather than [^{3}H]thymidine incorporation, avoiding potential problems of interpretation due to keratinocyte catabolism of thymidine.[22] TGF-β1 caused a marked (four to fivefold) and significant ($p < 0.01$) inhibition of proliferation was observed 6 days after treatment in both the normal and psoriatic keratinocytes. However, there was no evident or statistically significant ($p > .01$) difference between the antiproliferative responses of the two groups (compare solid lines, FIG. 3).

To further evaluate the lack of differential responses of normal and psoriatic keratinocytes to TGF-β1, we compared the ability of these cells to bind purified, radioio-

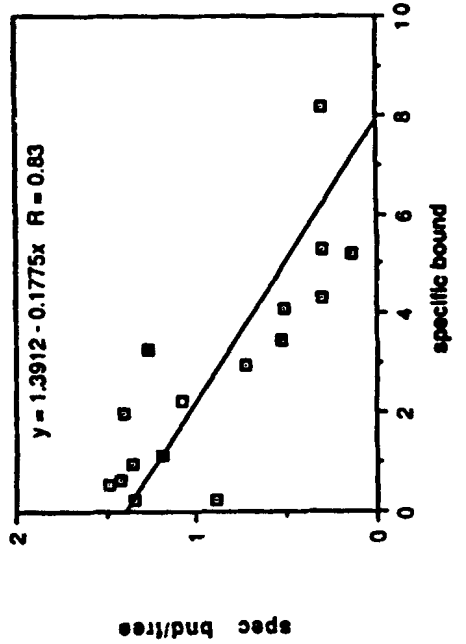
dinated TGF-β1 by Scatchard analysis. FIGURE 4 displays the binding data obtained from representative individual cultures of keratinocytes derived from normal (upper panel), uninvolved psoriatic (middle panel), and involved psoriatic epidermis (lower panel). Similar data were used to calculate binding constants (K_d) and the number of sites per cell for an additional three normal and two involved psoriatic keratinocyte cultures. These results are summarized in TABLE 1. The data indicate that normal, uninvolved, and lesional keratinocytes are similar in terms of binding affinities and number of binding sites per cell for TGF-β1.

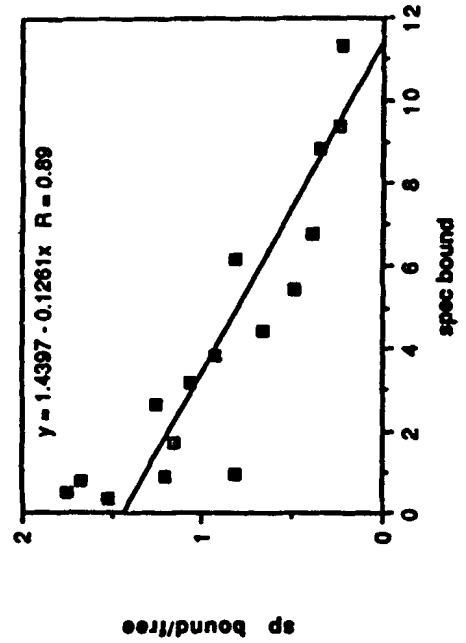
Three forms of the TGF-β1 receptor that differ in molecular weight have been detected in fibroblasts and other cell types by crosslinking ^{125}I-labeled TGF-β to the receptor with disuccinimidyl suberate (DSS).[21,22] We have applied this technique to keratinocytes to determine whether normal and psoriatic keratinocytes differ in terms of receptor molecular weight profiles (FIG. 5). Normal and psoriatic keratinocytes displayed an unusual receptor profile, displaying a closely spaced triplet of bands corresponding to receptor-TGF-β1 complexes in the 65 K molecular weight range (lanes 1 and 3, FIG. 5). In comparison, 3T3 fibroblasts displayed bands corresponding to receptor complexes at 65, 90, and 280 K (lane 5) as previously reported.[21,22] All bands were effectively competed by addition of excess (20 nM) unlabeled TGF-β1 to the crosslinking reaction (lanes 2, 4, and 6). However, there was no clear difference between the receptor crosslinking patterns of normal and psoriatic keratinocytes. Increasing concentrations of unlabeled TGF-β1 reduced labeling of all three bands of the triplet to a similar extent, indicating that each molecular weight form of the keratinocyte receptor bound TGF-β1 with comparable efficiency (FIG. 6).

DISCUSSION

Increased keratinocyte proliferation in psoriasis[2] could be due either to increased production of or sensitivity to factor(s) that stimulate growth, or to decreased production of or sensitivity to factor(s) that inhibit growth. We have shown markedly increased expression of TGF-α in psoriatic lesions, while expression of TGF-β1 mRNA was similar in normal epidermis and psoriatic lesions.[7] Recently, Mansbridge and coworkers have reported focally increased expression of TGF-β1 in uninvolved and lesional psoriatic epidermis by immunocytochemical techniques.[24] Therefore, we have extended our previously reported studies to confirm by Northern blot analysis the similarity of TGF-β1 expression in normal and psoriatic skin (FIG. 1) and cultured keratinocytes (FIG. 2, third panel). Taken together, these results indicate that the increased TGF-β1 protein observed in psoriatic skin by Mansbridge and coworkers may be due to deposition of TGF-β1 synthesized elsewhere, differential activation of latent epidermally derived TGF-β1 precursors,[25] localized variations in TGF-β1 RNA not detectable by our techniques, or altered regulation of TGF-β1 translation in psoriatic skin. Moreover, it remains possible that other members of the TGF-β gene family[26–28] may be abnormally expressed in psoriatic skin.

Since TGF-β1 is expressed in psoriatic skin, it remained to test the hypothesis that the keratinocytes of psoriatic epidermis might have a diminished sensitivity to its antiproliferative effects. Coffey and colleagues have shown that TGF-β treatment reduces c-*myc* mRNA in the MK line of murine kertinocytes at a posttranscriptional level.[29] We find a similar reduction in steady-state c-*myc* mRNA levels in response to highly purified TGF-β1 in human keratinocytes (FIG. 2, top panel). TGF-β has also been shown





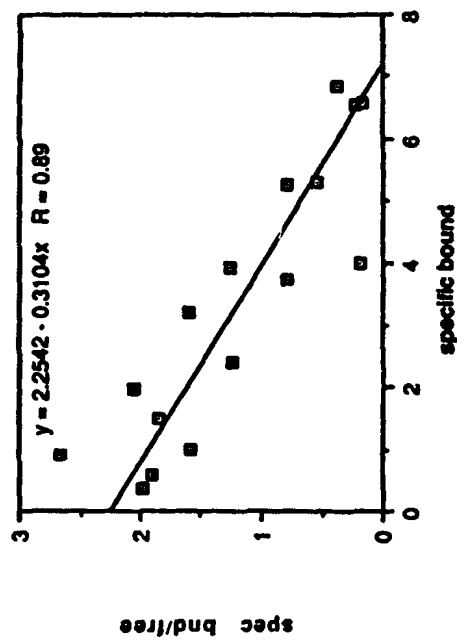


FIGURE 4. Scatchard analysis of TGF-β1 binding to normal (upper left panel), uninvolved (upper right panel), and lesional (lower left panel) psoriatic cultured keratinocytes.

TABLE 1. Transforming Growth Factor-β1 Receptor Binding and Affinity on Normal and Psoriatic (Lesional) Keratinocytes

Cell Sources	Number of TFG-β1 Receptors per Cell	Binding Affinity (K_d)
Control keratinocytes		
Normal adult	2052	6.402 pM
Normal adult	4091	8.285 pM
Normal adult	5497	10.246 pM
Normal adult	6877	7.930 pM
Psoriatic keratinocytes		
Involved lesion	7631	10.060 pM
Involved lesion	4374	3.222 pM
Involved lesion	3563	4.230 pM
Uninvolved lesion	4721	5.634 pM

to markedly induce the expression of PAI-1 mRNA in human carcinoma, glioblastoma, and fibroblast cell lines.[30,31] However, this is the first report of such a response in a human primary culture system (FIG. 2, second panel). Neither this response nor the reduction of c-*myc* mRNA after TGF-β1 treatment was significantly different in normal and psoriatic keratinocytes, indicating that both cell types are comparably responsive to TGF-β1 at the concentration tested (1 ng/ml, 40 pM).

As shown by Wilke, Pittelkow, and coworkers,[32] TGF-β induces a reversible state of growth arrest without induction of terminal differentiation in cultured neonatal human keratinocytes. We have extended these results to adult human keratinocytes derived from normal and psoriatic epidermis (FIG. 3), and find that normal and psoriatic cells do not differ in antiproliferative responsiveness to TGF-β1 (1 ng/ml, 40 pM).

Consistent with these findings, Scatchard analysis of TGF-β1 binding revealed a comparable range of values for binding constants (K_d) and numbers of binding sites per cell for normal and psoriatic keratinocytes (FIG. 4, TABLE 1). The K_d values are substantially higher and the numbers of sites per cell are substantially lower than those reported for neonatal foreskin keratinocytes by Shipley and coworkers (428 pM, 35,000 sites/cell).[11] However, these differences may be accounted for by the fact that the latter experiments were performed at room temperature, while our experiments were performed at 4°C.

Receptor crosslinking studies using ^{125}I-labeled TGF-β1 and DSS yielded the unexpected result that both normal and psoriatic keratinocytes displayed a prominent triplet of bands at approximately 65 kD, and very little signal at 90 and 280 kD as observed in 3T3 fibroblasts (FIG. 5). Unlabeled TGF-β1 competed equally well for crosslinking of all three bands of the 65 kD triplet (FIG. 6), suggesting that each form of the 53 kD receptor bind TGF-β1 with comparable affinity. Our finding that keratinocytes express the low molecular weight form(s) of the receptor is consistent with its recent identification as the receptor conferring growth inhibition in mink lung epithelial cells.[34] This form of the TGF-β receptor has 10- to 30-fold higher affinity for TGF-β1 than for TGF-β2.[34] Thus, it is puzzling that cultured keratinocytes and mink lung epithelial cells display very similar growth inhibitory responses to TGF-β1 and -β2 (M. Pittelkow, personal communication).[34]

Our data indicate that keratinocytes derived from psoriatic lesions are as respon-

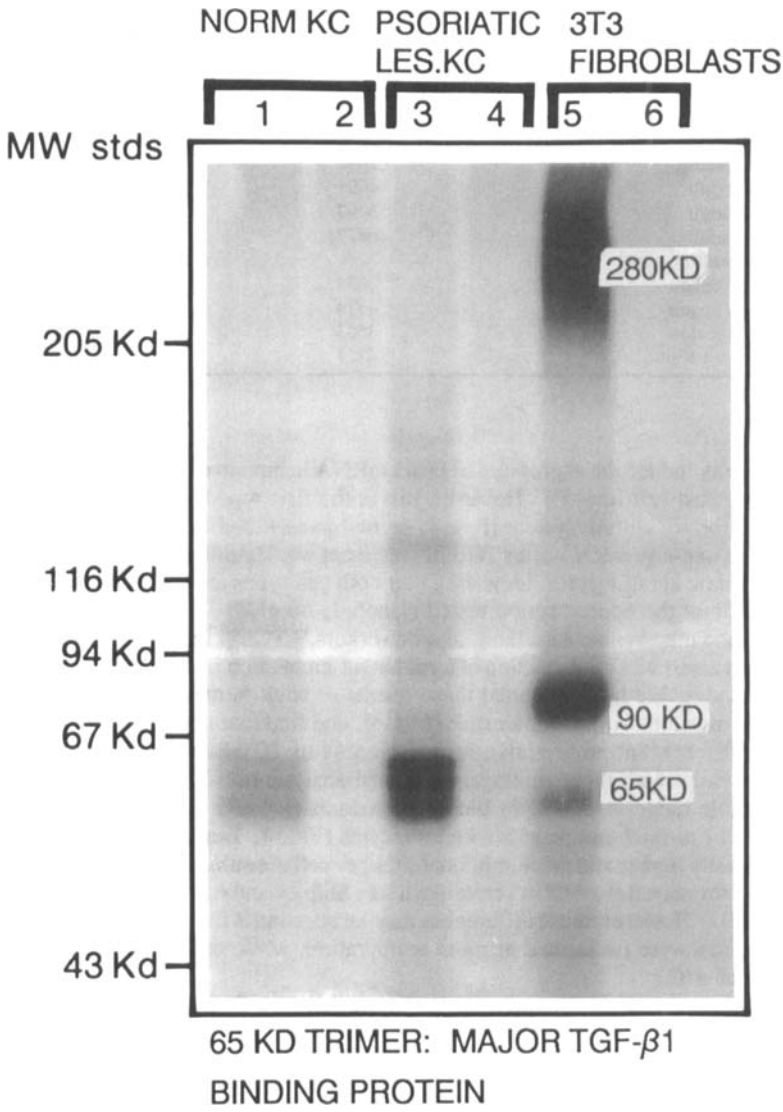


FIGURE 5. Receptor crosslinking analysis of keratinocyte and fibroblast TGF-β1 receptors. Light-colored line at 94 kD is an artifact.

sive to TGF-β1 as are normal keratinocytes, and that TGF-β1 can have a potent and direct antiproliferative effect on psoriatic keratinocytes. These results imply that TGF-β could be an effective antipsoriatic agent, given an effective means of drug delivery. Since TGF-β increases collagen production by dermal fibroblasts potentially leading to fibrosis,[35] it may be important to deliver TGF-β directly to the epidermis by topical means rather than by intradermal injection. Systemic delivery of TGF-β is compli-

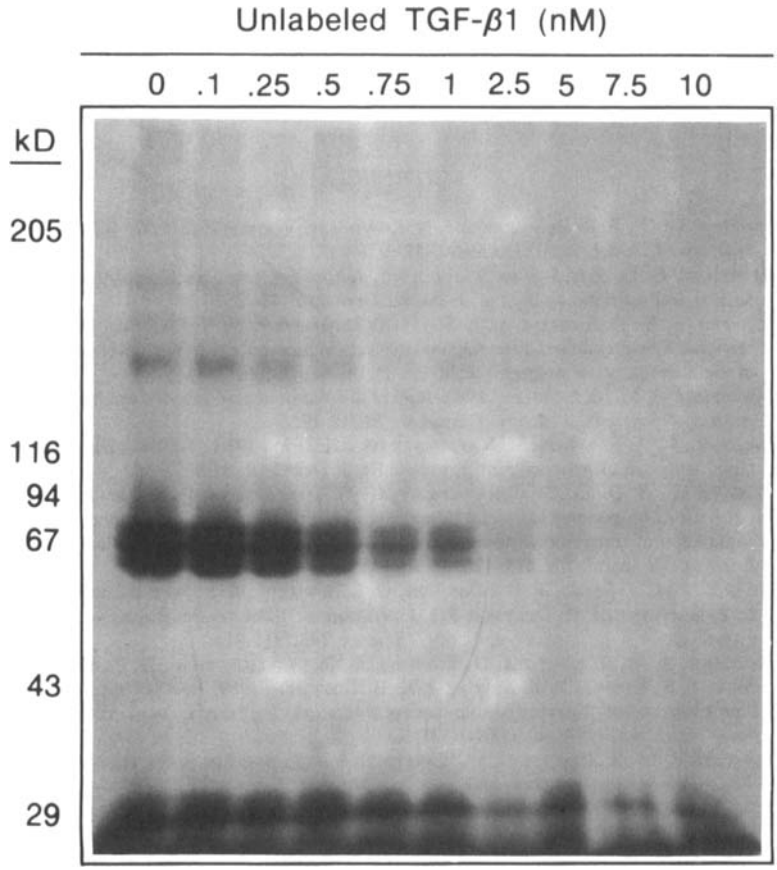


FIGURE 6. Competition of TGF-β1 receptor crosslinking with unlabeled TGF-β1.

cated by the potential for pleiotypic effects on a variety of cell types, and by its rapid hepatic clearance.[36] Further work on the use of penetration enhancers to promote epidermal delivery of TGF-β is clearly warranted.

Finally, it is very important that the proliferative response of psoriatic epidermis may be intimately related to the presence of epidermal and dermal T cells, monocytes, and macrophages in psoriatic lesions.[5,6] The dramatic response of psoriasis to low doses of cyclosporin A (CsA) underscores the potential role of T cells in this process, since CsA inhibits T cell function at doses far lower than those required to suppress keratinocyte proliferation directly *in vitro*.[37] Thus, CsA inhibits T cell release of γ-IFN, which we have recently shown to be capable of inducing TGF-α in keratinocytes.[17] TGF-β is a potent inhibitor of IL-1-independent thymocyte proliferation[21,38] and IL-2-dependent T lymphocyte proliferation.[39] Nickoloff and colleagues have demonstrated that keratinocyte-derived lymphocyte inhibitory factor activity contains immunoreactive TGF-β.[40] Whether T lymphocytes will prove to be a major site of TGF-β antipsoriatic action is an interesting question for future studies.

[NOTE ADDED IN PROOF: A 2kb PAI-1 transcript was detected in normal and psoriatic epidermal RNA by blot hybridization. PAI-1 mRNA levels were not significantly increased or decreased in psoriatic lesions. Since PAI-1 is strongly induced by TGF-β1 in keratinocytes *in vitro*, these results suggest that TGF-β1 activity is not increased or decreased in psoriatic epidermis.]

REFERENCES

1. KRUEGER, G. G., P. R. BERGSTRESSER, N. LOWE, J. J. VOORHEES & G. D. WEINSTEIN. 1984. Psoriasis. J. Amer. Acad. Dermatol. **11:** 937–947.
2. WEINSTEIN, G. D. & E. J. VAN SCOTT. 1965. Autoradiographic analysis of turnover times of normal and psoriatic epidermis. J. Invest. Dermatol. **45:** 257.
3. BERNARD, B. A., D. ASSELINEAU, L. SCHAFFAR-DESHAYES & M. Y. DARMON. 1988. Abnormal sequence of expression of differentiation markers in psoriatic epidermis: inversion of two steps in the differentiation program? J. Invest. Dermatol. **90:** 801–805.
4. BRAVERMAN, I. M., & J. SIBLEY. 1982. Role of the microcirculation in the treatment and pathogenesis of psoriasis. J. Invest. Dermatol. **78:** 12–17.
5. BAKER, B. S., A. F. SWAIN, H. VALDIMARSSON & L. FRY. 1984. T-cell subpopulations in the blood and skin of patients with psoriasis. Br. J. Dermatol. **110:** 37–44.
6. HAMMAR, H., S.-Q. GU, A. JOHANNESSON, K.-G. SUNDKVIST & P. BIBERFELD. 1984. Subpopulations of mononuclear cells in microscopic lesions of psoriatic patients. Selective accumulation of suppressor/cytotoxic T cells in epidermis during the evolution of the lesion. J. Invest. Dermatol. **83:** 416–420.
7. ELDER, J. T., G. J. FISHER, P. B. LINDQUIST, G. L. BENNETT, M. R. PITTELKOW, R. J. COFFEY, L. ELLINGSWORTH, R. DERYNCK & J. J. VOORHEES. 1989. Overexpression of transforming growth factor α in psoriatic epidermis. Science **243:** 811–814.
8. GROSSMAN, R. M., J. KRUEGER, D. YOURISH, A. GRANELLI-PIPERNO, D. P. MURPHY, L. T. MAY, T. S. KUPPER, P. B. SEHGAL & A. B. GOTTLIEB. 1989. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc. Natl. Acad. Sci. USA **86:** 6367–6371.
9. SPORN, M. B., A. B. ROBERTS, L. M. WAKEFIELD & R. K. ASSOIAN. 1986. Transforming growth factor-β: Biological function and chemical structure. Science **233:** 532–534.
10. KESKI-OJA, J. & H. L. MOSES. 1987. Growth inhibitory polypeptides in the regulation of cell proliferation. Med. Biol. **65:** 13–20.
11. SHIPLEY, G. D., M. R. PITTELKOW, J. J. WILLE, R. E. SCOTT & H. L. MOSES. 1986. Reversible inhibition of normal human prokeratinocyte proliferation by type β transforming growth factor-growth inhibitor in serum-free medium. Cancer Res. **46:** 2068–2071.
12. WILKE, M. S., B. M. HSU, J. J. WILLE, M. R. PITTELKOW & R. E. SCOTT. 1988. Biologic mechanisms for the regulation of normal human keratinocyte proliferation and differentiation. Am. J. Pathol. **131:** 171–181.
13. ELLINGWORTH, L. R., J. E. BRENNAN, K. FOK, D. M. ROSEN, H. BENTZ, K. A. PIEZ & S. M. SEYEDIN. 1986. Antibodies to the N-terminal portion of cartilage-inducing factor A and transforming growth factor β. J. Biol. Chem. **261:** 12362–12367.
14. FROLIK, C., L. WAKEFIELD, D. SMITH & M. SPORN. 1984. Characterization of a membrane receptor for transforming growth factor-β in normal rat kidney fibroblasts. J. Biol. Chem. **259:** 10995–11000.
15. WILLE, J. J., M. R. PITTELKOW, G. R. SHIPLEY & R. E. SCOTT. 1984. Integrated Control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: clonal analyses, growth kinetics, and cell cycle studies. J. Cell. Physiol. **121:** 31–44.
16. BOYCE, S. T. & R. G. HAM. Normal human epidermal keratinocytes. *In* In Vitro Models for Cancer Research. Vol. III. M. M. Webber & L. I. Sekely, Eds.: 245–274. CRC Press. Boca Raton, FL.
17. NICKOLOFF, B. J., R. S. MITRA, J. T. ELDER, G. J. FISHER & J. J. VOORHEES. 1989. Decreased growth inhibition by recombinant gamma interferon is associated with increased transforming growth factor-α production in keratinocytes cultured from psoriatic lesions. Br. J. Dermatol. **121:** 1–14.

18. ELDER, J. T., A. K. GUPTA, G. J. FISHER & J. J. VOORHEES. 1988. Cyclosporine inhibits ornithine decarboxylase gene expression and acute inflammation in response to phorbol ester treatment of hairless mouse skin. Transplant Proc. **20:** 95–104.
19. DERYNCK, R., J. A. JARRETT, E. Y. CHEN, D. H. EATON & J. R. BELL, R. K. ASSOIAN, A. B. ROBERTS, M. B. SPORN & D. V. GOEDDEL. 1985. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature **316:** 701–705.
20. GINSBURG, D., R. ZEHEB, A. Y. YANG, U. M. RAFFERTY, P. A. ANDREASEN, L. NIELSEN, K. DANO, R. V. LEBO & T. D. GELEHRTER. cDNA cloning of human plasminogen activator-inhibitor from endothelial cells. J Clin Invest **78:** 1673–1680.
21. ELLINGSWORTH, L. R., D. NAKAYAMA, P. SEGARINI, J. DASCH, P. CARRILLO & W. WAEGELL. Transforming growth factor-βs are equipotent growth inhibitors of interleukin-1-induced thymocyte proliferation. Cell. Immun. **114:** 41–54.
22. KIMCHI, A., X.-F. WANG, R. A. WEINBERG, S. CHEIFETZ & J. MASSAGUÉ. 1988. Absence of TGF-β receptors and growth inhibitory responses in retinoblastoma cells. Science **240:** 196–199.
23. SCHWARTZ, P. M., L. C. KUGELMAN, Y. COIFMAN, L. M. HOUGH & L. M. MILSTONE. 1988. Human keratinocytes catabolize thymidine. J. Invest. Dermatol. **90:** 8–12.
24. KANE, C. J. M., P. C. HANAWALT, A. M. KNAPP & J. N. MANSBRIDGE. 1989. Immunohistochemical localization of transforming growth factor-β protein in psoriasis. J. Invest. Dermatol. **92A:** 455.
25. WAKEFIELD, L. M., D. M. SMITH, K. C. FLANDERS & M. B. SPORN. 1988. Latent transforming growth factor-β from human platelets. J. Biol. Chem. **263:** 7646–7654.
26. MASSAGUE, J. 1987. The TGF-β family of growth and differentiation factors. Cell **49:** 437–438.
27. DERYNCK, R., P. B. LINDQUIST, A. LEE, D. WEN, J. TAMM, J. L. GRAYCAR, L. RHEE, A. J. MASON, D. A. MILLER, R. J. COFFEY, H. L. MOSES & E. Y. CHEN. 1988. A new type of transforming growth factor-β, TGF-β3. EMBO J. **7:** 3737–3743.
28. JAKOWLEW, S. B., P. J. DILLARD, M. B. SPORN & A. B. ROBERTS. 1988. Complementary deoxyribonucleic acid cloning of a messenger ribonucleic acid encoding transforming growth factor β4 from chicken embryo chondrocytes. Mol. Endo. **2:** 1186–1195.
29. COFFEY, R. J., C. C. BASCOM, N. J. SPIES, R. GRAVES-DEAL, B. E. WEISSMAN & H. L. MOSES. 1988. Selective inhibition of growth-related gene expression in murine keratinocytes by transforming growth factor β. Mol. Cell Biol. **8:** 3088–3093.
30. HELSETH, E., A. DALEN, G. UNSGAARD, T. SKANDSEN, J. GRONDAHL-HANSEN & L. R. LUND. 1988. Transforming growth factor-β1 is a potent inducer of plasminogen activator inhibitor type-1 in human glioblastoma and carcinoma cell lines. Acta Pathol. Microbiol. Immunol. Scand. **96:** 845–849.
31. KESKI-OJA, J., R. RAGHOW, M. SAWDEY, D. J. LOSKUTOFF, A. E. POSTLETHWAITE, A. H. KANG & H. L. MOSES. 1988. Regulation of mRNAs for type-1 plasminogen activator inhibitor, fibronectin, and type I procollagen by transforming growth factor-β. J. Biol. Chem. **263:** 3111–3115.
32. WILKE, M. S., B. M. HSU & R. E. SCOTT. 1988. Two subtypes of reversible cell cycle restriction points exist in cultured normal human kertinocyte progenitor cells. Lab. Invest. **58:** 660–666.
33. ELDER, J. T., A. TAVAKKOL, S. B. KLEIN, M. E. ZEIGLER, M. WICHA & J. J. VOORHEES. 1990. Protooncogene expression in normal and psoriatic skin. J. Invest. Dermatol. **94:** 19–25.
34. BOYD, F. T. & J. MASSAGUE. 1989. Transforming growth factor-β inhibition of epithelial cell proliferation linked to the expression of a 53-kDa membrane receptor. J. Biol. Chem. **264:** 2272–2278.
35. MUSTOE, T. A ., G. F. PIERCE, A. THOMASON, P. GRAMATES, M. B. SPORN & T. F. DEUEL. 1987. Accelerated healing of incisional wounds in rats induced by transforming growth factor-β. Science **237:** 1333–1336.
36. COFFEY, R. J., L. J. KOST, R. M. LYONS, H. L. MOSES & N. F. LARUSSO. 1987. Hepatic processing of transforming growth factor β in the rat. J. Clin. Invest. **80:** 750–757.
37. FISHER, G. J., E. A. DUELL, B. J. NICKOLOFF, T. M. ANNESLEY, J. K. KOWALKE, C. N. ELLIS & J. J. VOORHEES. 1988. Levels of cyclosporin in epidermis of treated psoriasis patients differentially inhibit growth of keratinocytes cultured in serum free versus serum containing media. J. Invest. Dermatol. **91:** 142–146.
38. WAHL, S. M., D. A. HUNT, H. L. WONG, S. DOUGHERTY, N. MCCARTNEY-FRANCIS, L. M.

WAHL, L. ELLINGSWORTH, J. A. SCHMIDT, G. HALL, A. B. ROBERTS & M. B. SPORN. Transforming growth factor-β is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. J. Immunol. **140:** 3026–3032.

39. KEHRL, J. H., L. M. WAKEFIELD, A. B. ROBERTS, S. JAKOWLEW, M. ALVAREZ-MON, R. DERYNCK, M. B. SPORN & A. S. FAUCI. 1986. Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. J. Exp. Med. **163:** 1037–1050.
40. NICKOLOFF, B. J. & R. S. MITRA. 1988. Transforming growth factor-beta is a keratinocyte-derived lymphocyte inhibitory factor. J. Invest. Dermatol. **90A:** 592.