pine blocks Ca^{2+} currents in hippocampal neurons (7). Calcium concentration is a critical factor mediating K^+ currents that are decreased in hippocampal neurons from young animals conditioned to make the eyeblink response (3). (iii) Calcium-activated enzymes (Ca^{2+}-activated neutral protease and protein kinase C) have been implicated in regulating specific intracellular mechanisms correlated with learning and memory in young animals (15). These enzymes would not be expected to function optimally in aging brain in which endogenous intracellular Ca^{2+} levels are abnormal. These findings together with our present data suggest that Ca^{2+} antagonists such as nimodipine could ameliorate learning deficits in aging animals.

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11. Each group consisted of one male and five females. Aging rabbits were retired breeders. (Rabbits can live to 8 years of age.) The onset of age-related learning impairments begins at 30 to 32 months in rabbits. These "aging" or "middle aged" animals show learning and memory impairments comparable to old rabbits (*10*, and references therein). In agreement with those reports, we describe our subjects (32 to 48 months) as "aging." Animals were anesthetized with xylazine (6.0 mg/kg, intramuscular) and then injected with ketamine hydrochloride (45 mg/kg, intramuscular). Lidocaine hydrochloride (4.0 mg/kg) was injected into the incision areas. Animals were then chronically cannulated within the right posterior jugular vein. Behavioral testing was begun 48 hours later. Experimental infusions were made with vehicle (polyethylene glycol 400) or nimodipine diluted in vehicle. Animals were individually housed in a colony maintained on a 12:12 hour light-dark cycle with free access to food and water.
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Overexpression of Transforming Growth Factor α in Psoriatic Epidermis

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Transforming growth factor α (TGF-α) is produced by and required for the growth of epithelial cells and is angiogenic in vivo. Since epidermal hyperplasia and angiogenesis are hallmarks of psoriasis, TGF-α gene expression was analyzed in epidermal biopsies of normal and psoriatic skin. TGF-α messenger RNA and protein are much more abundant in lesional psoriatic epidermis than in normal-appearing skin of psoriatic patients or in normal epidermis. In contrast, messenger RNA levels of transforming growth factor β1 (TGF-β1), which inhibits epithelial cell growth, are not significantly different in normal, uninvolved, and lesional psoriatic epidermis. Thus, psoriatic epidermal hyperplasia may involve increased expression of a keratinocyte mitogen (TGF-α) rather than deficient expression of a growth inhibitor (TGF-β1).

PSORIASIS IS A COMMON SKIN DISease in which epidermal hyperproliferation is prominent (*1*). Other characteristic features of psoriasis include capillary elongation and dilatation and the presence of acute and chronic inflammatory cells of the dermis and epidermis (*2*). The recurrent and fluctuating nature of psoriasis is consistent with abnormally unstable regulation of a nonmalignant pattern of epidermal growth, vascular alterations, and dermal inflammation normally observed in the healing of wounded skin (*3*). Identification of factors capable of coordinately directing these responses, their cellular sources, and their cellular and molecular targets could provide important clues into the pathogenesis of psoriasis and other hyperplastic skin diseases and the process of wound healing.

TGF-α is a candidate for direct regulation of the epidermal and angiogenic components of the inflammatory hyperplastic response in psoriasis. TGF-α is structurally related to epidermal growth factor (EGF) and interacts with the same receptor as EGF (*4*). Both molecules are potent positive regulators of epithelial cell growth (*5, 6*), but TGF-α appears to be more potent than EGF in inducing mitosis and migration of human skin keratinocytes (*6*). Since human skin and cultured normal human keratinocytes produce TGF-α (*5*), its overexpression in keratinocytes could be responsible for the initiation or the maintenance of epidermal hyperproliferation in a psoriatic lesion. TGF-α has angiogenic activity in vivo surpassing that of EGF (*7*). Since the dermoepidermal junction is permeable to molecules the size of TGF-α (*8*), the marked dermal angiogenic response characteristic of psoriasis could be mediated by overproduction of TGF-α by keratinocytes. This would be consistent with the observation that the epidermis is much more potent than the dermis as a source of angiogenic activity (*9*).

We evaluated the expression of TGF-α at the levels of steady-state mRNA and protein in normal epidermis and in the normal-appearing (uninvolved) epidermis and lesional epidermis of patients with psoriasis. Since TGF-β gene expression occurs in human keratinocytes (*10*) and in phorbol ester–treated mouse skin (*11*), and since TGF-β can antagonize the growth-promoting effects of EGF and TGF-α in human and murine keratinocytes (*10*), we also assayed the expression of the TGF-β1 gene in normal and psoriatic skin.

Samples of epidermal tissue were obtained by keratome biopsy (*12*) from normal

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volunteers and from unpaired samples of psoriatic skin lesions (referred to as involved psoriatic epidermis) or the normal-appearing (uninvolved) skin of psoriatic patients. Statistical analyses were performed by one-way analysis of variance with Scheffe's correction for multiple comparisons and a two-tailed hypothesis.

An RNA blot (*13*) of normal and involved psoriatic epidermal total RNA probed with a TGF-α cDNA insert (*14*) is shown in Fig. 1. Two bands, one migrating at 4.5 to 4.8 kb and the other at approximately 2 kb (solid arrows) are much more intense in nine of the ten RNA samples from psoriatic lesions than in any samples derived from normal epidermis (Fig. 1A). These bands correspond to TGF-α–specific polyadenylated [poly(A)$^+$] RNAs and not to cross-hybridization to the 28*S* and 18*S* ribosomal RNA, as shown by the presence of identically sized bands in poly(A)$^+$ RNA from psoriatic lesions (Fig. 1B) and by the much lower signal intensity in total RNA from normal epidermis (Fig. 1A). An additional 1.0-kb band in the psoriatic total RNA samples (open arrow) was not visible in poly(A)$^+$ RNA and could represent an artifact or RNA degradation product. TGF-α and TGF-β1 mRNA levels are compared in slot blots of poly(A)$^+$ RNA and Northern blots of total RNA isolated from normal, uninvolved, and involved psoriatic epidermis in Fig. 1, C and D. In both experiments, TGF-α–specific hybridization is detected clearly only in the lesional psoriatic samples, whereas the TGF-β1 mRNA signal is clearly detectable in all three groups. Nonspecific cross-hybridization of the TGF-β1 probe (*14*) to the 28*S* RNA was observed at 42°C but was minimized by raising the hybridization temperature to 44°C (the 28*S* RNA signal is absent in Fig. 1D).

We quantitated TGF-α and TGF-β1 mRNA in total and poly(A)$^+$ RNA isolated from normal, uninvolved, and involved psoriatic skin by laser scanning densitometry of slot blots (Fig. 2). TGF-α mRNA in uninvolved psoriatic epidermis was not significantly different from that in normal epidermis. In involved psoriatic epidermis, however, TGF-α mRNA was increased 4.4-fold in total RNA and 5.7-fold in poly(A)$^+$ RNA, relative to values in normal controls ($P < 0.001$). In contrast, TGF-β1 mRNA was not significantly different in normal, uninvolved psoriatic, and involved psoriatic epidermis.

We next measured TGF-α and TGF-β1 protein levels in acid-ethanol extracts of normal, uninvolved psoriatic, and involved psoriatic epidermal homogenates by means of sensitive and specific immunoassays (*15*). TGF-α immunoreactive protein was not detected (less than 80 pg per keratome biopsy; minimum wet weight 100 mg) in 7 of 11 extracts of normal epidermis and in 11 of 14 extracts of uninvolved psoriatic epidermis. In the remaining normal and uninvolved samples, only small quantities of TGF-α were found (Fig. 3, A and B). In contrast, TGF-α was found in 12 of 13 psoriatic epidermal extracts, averaging 17.2 ng per gram of protein (Fig. 3A) and 1.1 ng per milligram of DNA (Fig. 3B). Thus, extractable TGF-α was increased at least 6-fold on a protein basis and 6.4-fold on a DNA basis in involved psoriatic relative to amounts present in normal epidermal extracts. The actual difference between normal and involved epidermis is probably higher, since samples in which TGF-α was undetectable by an enzyme-linked immunosorbent assay were assigned their maximum possible value for this comparison. TGF-α immunoreactive protein, like TGF-α mRNA, did not differ detectably in uninvolved psoriatic and normal epidermis. Recovery of purified, exogenous TGF-α added to tissue homogenates before extraction averaged 47% and was independent of the ratio of added TGF-α to total extract protein.

TGF-β1 was detectable by radioimmunoassay (*15*) in acid-ethanol extracts of normal and involved psoriatic epidermal samples. However, we encountered a variable binding of added unlabeled or radioiodinated TGF-β1 to uncharacterized material in our epidermal extracts, precluding meaningful quantitation of the results. Low recoveries or the presence of interfering factors, or both, may also explain our frequent inability to detect TGF-β1 by bioassay (*15*). Thus, acid-ethanol extracts of 10 of 13 samples obtained by keratome biopsy, including normal, uninvolved, and involved psoriatic skin, failed to stimulate colony growth of normal rat kidney fibroblasts in soft agar.

These results demonstrate quantitatively that the TGF-α gene is overexpressed in lesional psoriatic epidermis (*16*) and show that TGF-α is a potential mediator of the epidermal and angiogenic components of

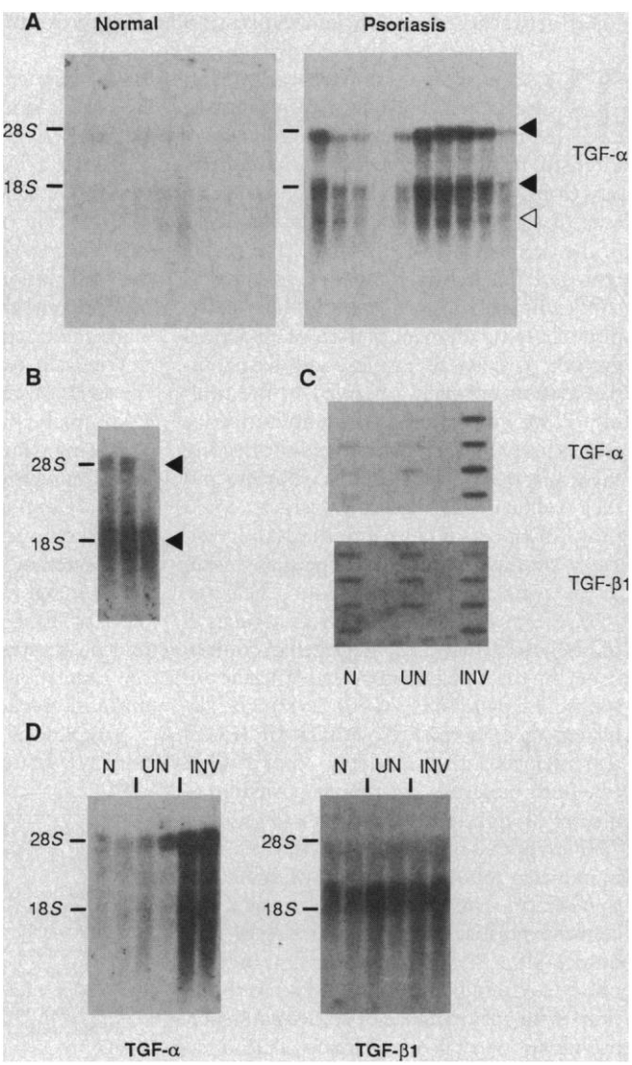

Fig. 1. Increased TGF-α mRNA in psoriatic lesions. (**A**) RNA blot hybridization analysis of total RNA (50 μg) extracted from normal and involved psoriatic epidermis. Each lane represents a different individual. The blot was hybridized with the TGF-α cDNA probe (*13, 14*). Mobilities of 28*S* and 18*S* ribosomal RNA are shown at left. (**B**) RNA blot of poly(A)$^+$ RNA (2 μg) extracted from psoriatic lesions (three patients) hybridized with the TGF-α probe. Closed arrows indicate TGF-α mRNAs. (**C**) RNA slot blot (*13*) of poly(A)$^+$ RNA (0.75 μg) isolated from four normal (N), three uninvolved (UN), and four involved psoriatic (INV) epidermal samples hybridized with the TGF-α or the TGF-β1 (14) probe. (**D**) Northern blot of total RNA (50 μg) isolated from normal, uninvolved, or involved psoriatic epidermis hybridized with the TGF-α or TGF-β1 cDNA probes.

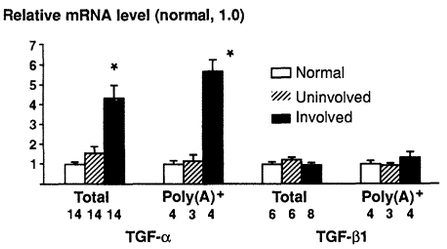

Fig. 2. Quantitation of TGF-α and TGF-β1 mRNA in total and poly(A)$^+$ RNA from normal, uninvolved, and involved psoriatic epidermis. Slot blots of total or poly(A)$^+$ RNA were scanned with a laser densitometer-integrator (LKB Ultroscan XL). The data were scaled by dividing by the mean of the normal group for each probe and type of RNA preparation. Therefore the bar heights represent the average level of TGF-α or TGF-β1 mRNA relative to normal epidermis. The number of samples is indicated below each bar. Error bars indicate standard error of the mean. Asterisks indicate statistically significant differences relative to normal controls ($P < 0.001$).

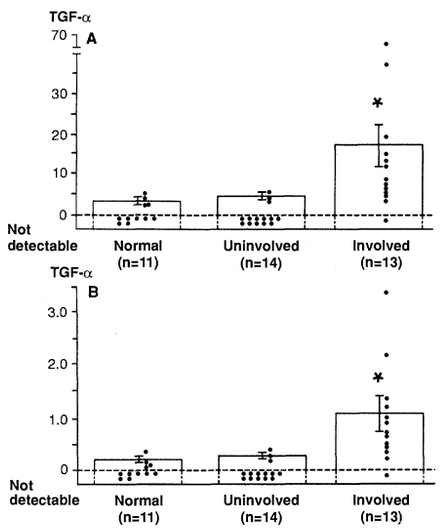

Fig. 3. TGF-α immunoreactive protein in normal, uninvolved, and involved psoriatic epidermis. Bar heights and error bars depict mean ± SEM for samples with detectable amounts only. Individual values are shown as solid dots. Statistical significance ($P < 0.02$) against the uninvolved psoriatic and normal groups is indicated by an asterisk above the bars. (**A**) Data are expressed in nanograms of TGF-α per gram of homogenate protein. (**B**) Data are expressed as nanograms of TGF-α per milligram of homogenate DNA.

the inflammatory hyperproliferative response. EGF, the homolog of TGF-α, is also capable of inducing keratinocyte proliferation and angiogenic responses (*5, 7*). However, we have been unable to detect EGF mRNA transcripts in normal or psoriatic epidermis or in cultured human keratinocytes, a result that is consistent with previous observations of very low levels of immunoreactive EGF in mouse skin (*17*). Since we find comparable levels of TGF-β1 mRNA in normal and psoriatic epidermis, and since we have been unable to demonstrate differential responsiveness of normal and psoriatic cultured keratinocytes to TGF-β1 treatment (*18*), our data lend little support to the hypothesis that decreased expression of the TGF-β1 gene or a loss of TGF-β1 responsiveness is coupled to unrestrained epidermal proliferation in psoriasis.

Amplification or rearrangement of the TGF-α gene could lead to increased expression of TGF-α mRNA in psoriatic epidermis. To test for these possibilities, we prepared DNA blots of Pst I–digested genomic DNA extracted from normal ($n = 5$), uninvolved ($n = 5$), and involved ($n = 5$) epidermis obtained by keratome biopsy. Each group yielded band patterns of equivalent intensity and identical fragment sizes when hybridized to the TGF-α cDNA probe (*19*), demonstrating that neither amplification nor gross rearrangement of the TGF-α gene is present in psoriatic lesions.

Nonkeratinocyte cell types such as macrophages, which are known to express TGF-α and TGF-β at wound sites in vivo and when activated in vitro (*20*), could contribute to the TGF-α mRNA and immunoreactive protein measured in the psoriatic samples. Psoriatic epidermis contains approximately 4% bone marrow–derived HLA-DR$^+$ cells consisting of T cells, antigen-presenting cells, and monocytes and macrophages (*21*). In addition, skin samples obtained by keratome biopsy contain small but variable quantities of endothelial cells, fibroblasts, polymorphonuclear leukocytes, mast cells, macrophages, and T cells as a result of dermal contamination. Our inability to detect significant increases in TGF-β1 mRNA in involved psoriatic epidermis in spite of large (four- to sixfold) increases in TGF-α mRNA and protein argues against macrophages being the primary source of epidermal TGF-α in the fully developed psoriatic lesions we analyzed. However, it remains possible that macrophage-derived TGF-α participates in the initiation and maintenance of psoriatic lesions. Early lesions of psoriasis display enlargement and tortuosity of dermal capillaries which soon become surrounded by mononuclear cells, largely macrophages and T cells with some mast cells. These changes precede the development of epidermal hyperplasia and are the last to disappear in resolving psoriatic lesions (*2*). By secretion of TGF-α, macrophages or other dermal mononuclear cells could initiate TGF-α autoinduction in keratinocytes (*5*) with concomitant hyperproliferation (*6*). TGF-α produced in amplified amounts by keratinocytes could diffuse back to the dermis, perpetuating and extending abnormalities in the dermal microvasculature (*2*). Whether TGF-α plays any role in the accumulation of dermal and epidermal T cells and macrophages in psoriasis (*2*) remains to be determined.

Psoriatic epidermis displays pleiotypic biochemical abnormalities related to the control of cellular proliferation and differentiation. Many of these abnormalities may be linked to activation of the EGF receptor tyrosine kinase by TGF-α (*22*). The EGF receptor tyrosine kinase is subject to negative regulation by protein kinase C (PKC)–mediated phosphorylation of Thr^{654} (*23*). PKC activity is decreased in chronic psoriatic lesions (*24*). Diminished PKC activity in psoriasis may release EGF receptor kinase from its normal state of negative regulation, resulting in the increased proliferation and TGF-α expression characteristic of psoriatic lesions. These concepts provide testable hypotheses for future studies of the role of TGF-α in the pathogenesis of psoriasis.

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14. Sources of plasmids used in this study: psp65Cl7N3 contains a human TGF-α cDNA insert as present in pTGF-C1 [R. Derynck *et al.*, *Cell* **38**, 287 (1984)]; pTGF-βas contains a 1.04-kb human TGF-β1 cDNA insert as present in λβCl [R. Derynck *et al.*, *Nature* **316**, 701 (1985)]; phEGF116 contains a 4.2-kb human EGF cDNA insert [G. I. Bell *et al.*, *Nucleic Acids. Res.* **14**, 8427 (1986)].
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Purification of Growth Hormone–Specific Transcription Factor GHF-1 Containing Homeobox

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Pituitary-specific expression of the growth hormone (GH) gene is governed by a transcription factor, GHF-1, that binds to two sites within its promoter. Recently, GHF-1 was shown to be a member of the homeobox family of DNA-binding proteins. An important question is whether GHF-1 controls the expression of other pituitary specific genes, such as prolactin (Prl), expressed in closely related cell types. To this end, GHF-1 was purified from extracts of GH- and Prl-expressing pituitary tumor cells and identified as a 33-kilodalton polypeptide. Although GHF-1 bound to and activated the GH promoter, it did not recognize the Prl promoter. However, at least one other factor in the same extracts, which was easily separated from GHF-1, bound to several sites within the Prl but not the GH promoter. Antibodies to GHF-1 did not react with the Prl binding activity. These results imply that the pituitary-specific expression of GH and Prl is governed by two distinct trans-acting factors.

THE PITUITARY-SPECIFIC EXPRESsion of the GH gene is due to the recognition of its promoter region by a specific transcription factor, GHF-1 (*1*). GHF-1 has, thus far, been detected only in GH-expressing cell types (*2–4*). Extinction of GH expression in somatic cell hybrids appears to be caused by repression of GHF-1 expression (*3*). However, when added to extracts of nonexpressing cells such as HeLa, GHF-1 activates the GH promoter (*1*). The analysis of recently isolated cDNA clones encoding GHF-1 has indicated that GHF-1 is a homeobox-containing protein (*4*) and therefore is a member of a large family of DNA-binding proteins that control development and differentiation (*5*). Immunohistological localization indicates that GHF-1 is expressed in cells of the somatotropic lineage in the anterior pituitary (*4*). GHF-1 may therefore be the major determinant specifying expression of GH in these cells.

An unresolved question is whether GHF-1 also controls expression of other anterior pituitary specific genes, such as prolactin (Prl). To address this question, we purified GHF-1 from whole cell extracts of pituitary tumor cells grown in suspension. These cells express both the GH and Prl genes (*6*).

A whole cell extract of GC cells (*7*) was fractionated by chromatography on heparin agarose and then analyzed on a Sephacryl S-300 gel filtration column (*8*). The GHF-1–enriched fractions were pooled and applied to a sequence-specific oligodeoxynucleotide-Sepharose column containing a high-affinity GHF-1 binding site (*9*). Purification of GHF-1 was monitored by deoxyribonuclease I (DNA I) footprinting. However, it was important to determine whether the transcriptional stimulatory activity of GHF-1 copurified with its DNA-binding activity. Furthermore, we wished to determine whether GHF-1 was the only pituitary cell–derived factor required for activating the GH promoter in extracts of nonexpressing HeLa cells. We therefore also monitored the transcriptional stimulatory activity of GHF-1 by adding a sample of each fraction to HeLa whole cell extract and measuring the level of transcripts initiated at the GH promoter by primer extension (*1, 10*). Each of the GHF-1–containing fractions stimulated initiation from the correct start site of the human GH (hGH) promoter in vitro, while having no effect on initiation from an adjacent nonspecific site (Fig. 1). Both start sites are used during transient expression of the hGH–chloramphenicol acetyltransferase (CAT) vector, although the physiological site is the dominant one (*2*). The ratio between the footprinting activity of GHF-1 (Table 1) and its ability to stimulate transcription was relatively unchanged during all three purification steps. Both activities eluted from the preparative S-300 column as a single peak corresponding to an apparent molecular mass of approximately 50 kD (*11*). A summary of the purification scheme is presented in Table 1.

SDS–polyacrylamide gel electrophoresis

Table 1. Purification of GHF-1.

Fraction	Protein (mg)	Volume (ml)	Units*	Yield (%)	Relative† purity
Whole cell extract	800	50	880,000	100	1
Heparin-agarose	250	30	750,000	85	3
S-300	27	54	450,000	51	17
DNA-affinity	0.21	10	150,000	17	700

*One footprinting unit is the amount of GHF-1 required for full protection of the proximal GH site with the use of a 5 ng of probe. †To determine relative purity, we assumed that the whole cell extract contained the same amount of GHF-1 as all of the heparin-agarose fractions, although it was never assayed directly by footprinting.

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