Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5237–5241, June 1993
Developmental Biology

Inhibition of skin development by overexpression of transforming growth factor β_1 in the epidermis of transgenic mice

(keratinocytes/growth factors/mitotic arrest/lethality)

KLAUS SELLHEYER*, JACKIE R. BICKENBACH*, JOSEPH A. ROTHNAGEL*, DONNIE BUNDMAN*, MARY ANN LONGLEY*, THOMAS KRIEG†, NANETTE S. ROCHE‡, ANITA B. ROBERTS‡, AND DENNIS R. ROOP*§

*Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX 77030; †Department of Dermatology, University of Cologne, Cologne, Germany; and ‡Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892

Communicated by Bert W. O'Malley, March 2, 1993 (received for review February 5, 1993)

ABSTRACT **To assess the effect of transforming growth factor β_1 on the skin *in vivo*, we have targeted its expression to the epidermis of transgenic mice. To ensure that active TGF-β_1 was expressed, we used a porcine TGF-β_1 cDNA with mutations of Cys-223 → Ser and Cys-225 → Ser, which allow constitutive activation. Mice expressing the mutant transforming growth factor β_1 transgene exhibited a marked phenotype at birth. The skin was very shiny and tautly stretched. These animals were rigid and appeared to be restricted in their ability to move and breathe; death occurred within 24 hr. Histologically, the most prominent features of the skin were a compact orthohyperkeratosis and a reduction in the number of hair follicles. Pulse-labeling studies with 5-bromodeoxyuridine demonstrated a marked reduction in the number of replicating cells in the epidermis and hair follicles. Thus, the macro- and microscopic appearance of these mice, as well as their neonatal lethality, most likely result from inhibition of normal skin development and suppression of epithelial cell proliferation by the overexpression of transforming growth factor β_1.**

Since the discovery of transforming growth factor β (TGF-β) more than a decade ago, it has become apparent that this family of closely related peptides is involved in a variety of biological processes, such as embryogenesis, carcinogenesis, wound healing, and immunomodulation (for reviews, see refs. 1 and 2). Three isoforms of TGF-β (TGF-β_1, TGF-β_2, and TGF-β_3) have been identified in mammals, and all are synthesized as pre/pro proteins and subsequently cleaved to yield the active C-terminal dimer. The remainder of the precursor, called the latency-associated peptide (LAP) is also dimeric and is secreted from cells in a noncovalent complex with the C-terminal dimer rendering it biologically inactive or "latent." Biologically active TGF-β results after dissociation from the LAP (1, 2). TGF-β often has seemingly opposite effects on different cell types within a given organ. With respect to the skin, *in vitro* studies have shown that TGF-β induces proliferation of dermal fibroblasts (3, 4) but arrests growth of epidermal keratinocytes (5–7). TGF-β induces fibrosis and angiogenesis when injected s.c. into newborn mice; however, no apparent effects were reported in the epidermis (8). To gain further insight into the biological function of TGF-β *in vivo*, several laboratories have used techniques of *in situ* hybridization and immunohistochemistry to examine the expression patterns of different isoforms during mouse development (9–14). Changes in the temporal and spatial distribution of RNA and protein have been seen for different isoforms in the skin, suggesting a role for these factors in cutaneous development. However, due to the complex mechanisms regulating transcription, translation, secretion, and activation of TGF-β, it is difficult to correlate these observations.

To obtain more direct evidence for involvement of TGF-β in skin development, we have targeted its expression to the epidermis of transgenic mice. Because over-expression of TGF-β at an early stage of embryogenesis could result in lethality *in utero*, we targeted expression with an epidermal-specific vector that is not expressed until day 15 of development (15). To ensure the expression of active TGF-β, we used a porcine TGF-β_1 cDNA with two site-specific mutations of Cys-223 → Ser and Cys-225 → Ser. These mutations are thought to preclude dimerization of the LAP protein and to interfere with its ability to complex with the C-terminal domain, resulting in the secretion of active TGF-β_1 (16). Here we report the generation of transgenic mice that express TGF-β_1 in the epidermis and exhibit a severe skin phenotype resulting in neonatal lethality. This study documents the role of TGF-β_1 as a potent inhibitor of epithelial-cell proliferation *in vivo*.

MATERIALS AND METHODS

Generation and Identification of Transgenic Mice. The HK1.TGF-β_1 transgene (Fig. 1*A*) was cut from the pGem3 plasmid with *Eco*RI and isolated by agarose gel electrophoresis and paper purification (NA45 paper, Schleicher & Schuell). The DNA was further purified by ultracentrifugation and microinjected into the pronuclei of mouse embryos obtained from strain ICR female mice mated to strain FVB males. After microinjection, 15–20 embryos were transferred to the oviduct of each ICR pseudo-pregnant foster mother, as described (15). After birth, transgenic pups were confirmed by PCR analysis of their DNA using oligonucleotides specific for the first intron of the expression vector (Fig. 1).

Isolation and Preparation of RNA. Skins from newborn transgenic and control mice were floated on 0.25% trypsin at 4°C for 18 hr, after which the epidermis and dermis were separated as independent sheets (18). Total RNA was isolated from the epidermis and dermis and assessed for HK1.TGF-β_1 expression by reverse transcriptase/PCR analysis as described (15). The specific oligonucleotides used for this analysis are shown in Fig. 1.

Immunohistochemistry. Back skin samples from transgenic and control mice were processed for immunohistochemical demonstration of TGF-β_1 using a described immunoperoxidase system (9). Three primary antibodies were used; two reacted with epitopes on the mature form of TGF-β_1, LC-(1–30), which stains intracellular TGF-β_1, and CC-(1–30),

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TGF-β, transforming growth factor β; BrdUrd, 5-bromodeoxyuridine; HK1, human keratin 1; K6, K10, K14, and K16, keratins 6, 10, 14, and 16, respectively; LAP, latency-associated peptide.

§To whom reprint requests should be addressed.

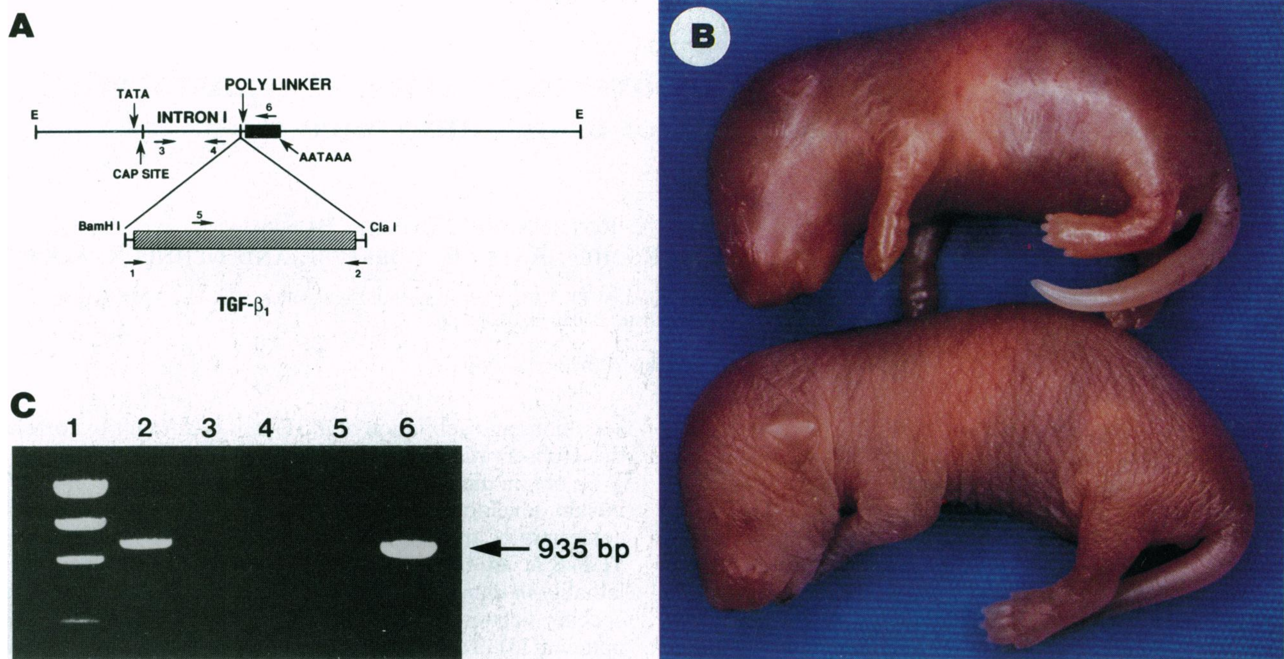

FIG. 1. Transgene construct, TGF-β_1 phenotype, and transgene expression. (*A*) Schematic showing structure of the HK1.TGF-β_1 transgene. Construction and characterization of the HK1 epidermal targeting vector have been described (15, 17). With a mutant derivative of porcine TGF-β_1 cDNA clone (16) as a template and oligonucleotides 1 and 2, a PCR product was synthesized and inserted into the *Bam*HI and *Cla* I sites of the vector polylinker. Oligonucleotides 3 and 4 are specific for the first intron and were used for screening transgenic mice. Oligonucleotide 6, which is specific for the 3' noncoding region of the vector was used to generate cDNA with reverse transcriptase, and oligonucleotides 5 and 6 were used to amplify the cDNA for the reverse transcriptase/PCR analysis. (*B*) Gross phenotypic morphology of a HK1.TGF-β_1 transgenic pup (*Upper*) with its normal littermate (*Lower*) 1 hr after birth. (*C*) Expression of HK1.TGF-β_1 transgene mRNA in phenotypic epidermis but not in dermis or control mice. Total RNA from epidermis or dermis was subjected to reverse transcriptase/PCR analysis. Lanes: 1, ØX174/*Hae* III markers; 2, transgenic epidermis; 3, control epidermis; 4, transgenic dermis; 5, control dermis; 6, pHK1.TGF-β_1 plasmid.

which stains extracellular TGF-β_1, and Karen, which reacts with the epitope represented by amino acids 267–278 of pre-pro-TGF-β_1 (9).

Immunofluorescence. Frozen sections from transgenic and control epidermis were incubated with rabbit anti-mouse antibodies to keratin 1 (K1), keratin 6 (K6), keratin 10 (K10), loricrin, and filaggrin; and with guinea pig anti-mouse antibody to keratin 14 (K14). The reactions were visualized by secondary antibodies fluorescein isothiocyanate-labeled anti-rabbit IgG and biotin-labeled anti-guinea pig IgG and streptavidin Texas red (see ref. 15 for details).

Light and EM. Back skin samples from transgenic and control mice were fixed in Carnoy's solution or 10% buffered formalin, and 5-μm sections were stained with hematoxylin/eosin. For hair follicle counts, the epidermis in every fifth section for a total of six sections was measured by using a micrometer, and the number of hair follicle cross sections was counted. For transmission electron microscopy, samples were fixed in glutaraldehyde and osmium tetroxide and embedded in Spurr's plastic; 60-nm sections were stained with uranyl acetate and lead citrate.

Bromodeoxyuridine (BrdUrd) Labeling. Back skin samples from transgenic and controls were incubated 4 hr at 37°C in medium containing BrdUrd at 20 nmol/ml, fixed in 70% cold ethanol, embedded in paraffin, and stained with an undiluted fluorescein isothiocyanate-conjugated monoclonal antibody to BrdUrd (Becton Dickinson), and counterstained with guinea pig anti-mouse K14 to visualize the epidermis and hair follicles.

RESULTS

TGF-β_1 Transgenic Mice Exhibit a Marked Skin Phenotype at Birth. The construct used to target expression of TGF-β_1 in transgenic mice, pHK1.TGF-β_1, is shown in Fig. 1*A*. The epidermal specific targeting vector was derived from the regulatory sequences of the human keratin 1 (HK1) gene, and its construction and characterization have been described (15, 17). The first pup to test positive for the HK1.TGF-β_1 transgene was found dead ≈15 hr after birth. Macro- and microscopic analyses indicated abnormalities in skin development and suggested that this may have led to lethality. To determine whether phenotypic pups were stillborn or died shortly after birth, we carefully monitored litters at birth and observed four live-born, phenotypic pups in subsequent litters. They were rigid and restricted in their ability to move and breathe. Due to the severity of this phenotype, these animals were sacrificed shortly after birth along with normal nontransgenic littermates. All four phenotypic pups tested positive for the transgene. Although there were slight variations in the phenotypic appearance of the four transgenic pups, they all exhibited gross morphological differences from their normal littermates (Fig. 1*B*). Their bodies were slightly smaller, as were their appendages, such as paws and ears. Their skin appeared shiny and tautly stretched and did not show normal dermatoglyphic patterns.

Expression of HK1.TGF-β_1 Transgene in the Epidermis. To demonstrate expression of HK1.TGF-β_1 in the epidermis, we isolated total RNA from the epidermis and dermis of phenotypic and control littermates. After preparing a cDNA template of these RNAs, specific oligonucleotide primers were used to amplify cDNAs corresponding to the 3' noncoding region of the HK1 vector (see Fig. 1*A*). The PCR cDNA band diagnostic for transcripts from the HK1 vector is 935 bp and could be produced from control pHK1.TGF-β_1 plasmid DNA (Fig. 1*C*). A PCR product of this size was also produced from phenotypic epidermal RNAs but not dermal RNAs. Control

epidermal and dermal RNAs were also negative, confirming specificity of the assay.

To detect expression of HK1.TGF-β_1 at the protein level, we performed immunohistochemical analysis with three antisera specific for TGF-β_1. No obvious differences in the staining pattern of TGF-β_1 were seen in phenotypic and control skins when antisera that primarily react with extracellular [anti-CC-(1–30)] or intracellular [anti-LC-(1–30)] forms of mature TGF-β_1 were used (data not shown). However, an antibody directed against an epitope on the LAP (Karen) exhibited more intense staining in the epidermis of transgenic animals as compared with control littermates (Fig. 2 *A* and *B*). The difference in staining intensity was most prominent in the spinous and granular layers of the epidermis. Comparable levels of TGF-β_1 were detected in the dermis of both animals. Because expression of the

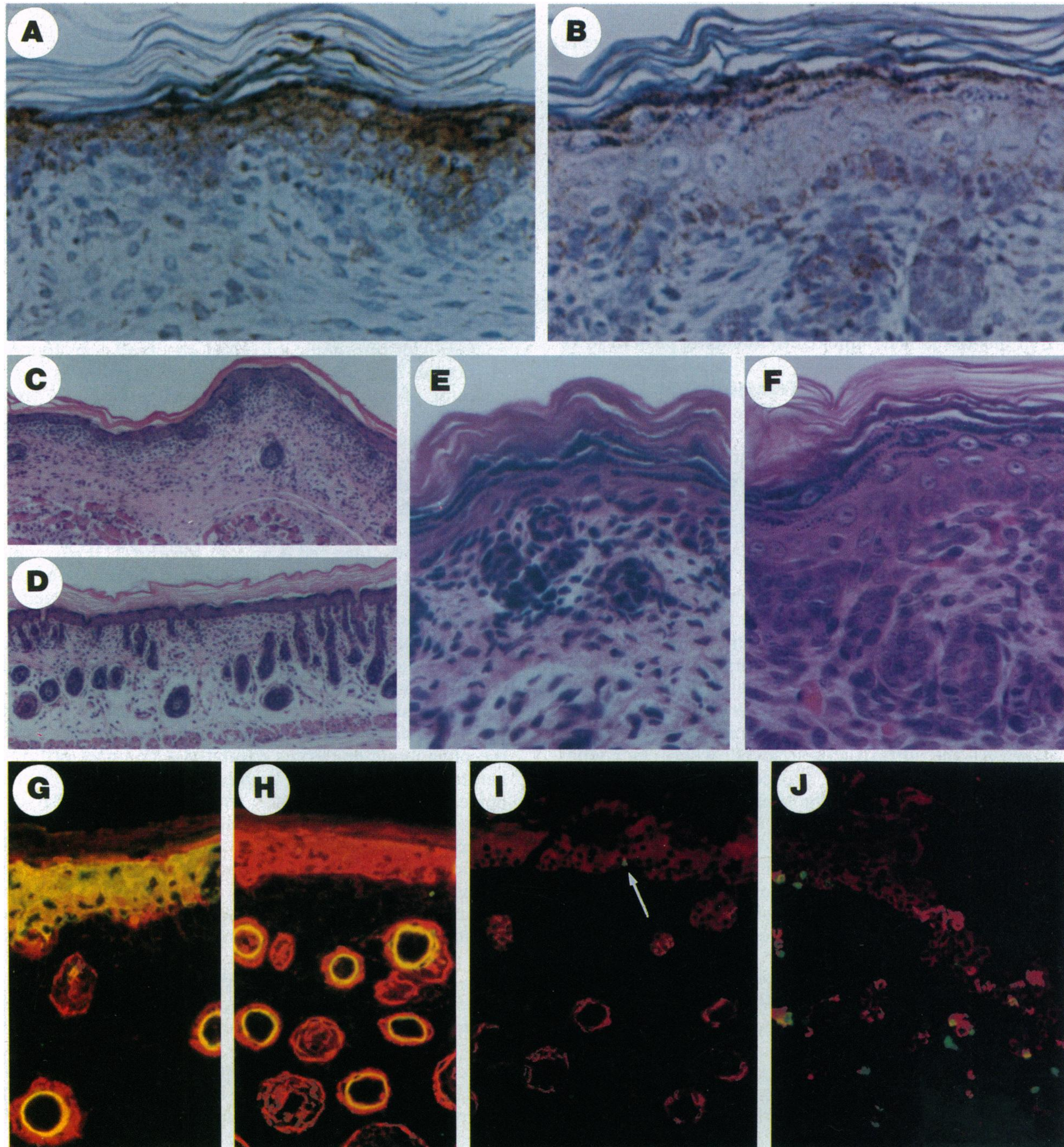

FIG. 2. Immunohistochemistry, histopathology, K6 expression, and BrdUrd labeling. Immunohistochemical staining of TGF-β_1 latency-associated peptide is more pronounced in the spinous and granular layers of the epidermis from transgenic (*A*) than from control (*B*) mice. Low magnification (×167) of hematoxylin/eosin-stained paraffin sections from transgenic skin (*C*) shows fewer hair follicles than a similar section from control skin (*D*). At higher magnification (×667) transgenic skin (*E*) shows a thinner epidermis and more compact orthohyperkeratosis than control skin (*F*). Marked interfollicular staining with antibody to K6 (green or yellow) is apparent in transgenic epidermis (*G*) as compared with controls (*H*). In both cases several hair follicles stain positive for K6. To show the epithelial portions of the sections, double-label immunofluorescence was done with antibody to K14 (red); areas containing both epitopes stain yellow. BrdUrd labeling (green or yellow) of transgenic skin (*I*) in organ culture showed almost a complete shut-down of DNA synthesis in the interfollicular and follicular epithelial cells, as compared with control skin (*J*). As described above, the sections were also double-labeled with K14 (red). Arrow in *I* denotes a single-labeled nucleus.

HK1.TGF-β_1 transgene was restricted to the epidermis at the RNA level, we assume that the TGF-β_1 detected in the dermis represents endogenous expression. We also observed a correlation between staining intensity and the gross pathological appearance of phenotypic skin, in that the most severely stretched skin reacted to the greatest extent with this antiserum (data not shown).

The discordance between staining for mature TGF-β_1, as detected by anti-CC-(1–30) or anti-LC-(1–30), and staining for the LAP in the epidermis, where the transgene is expressed, probably reflects the rapid internalization or sequestering of the mature active TGF-β, as expressed from this transgene construct. That is, latent TGF-β has a much longer half-life than does active TGF-β (19), presumably because association of mature TGF-β with the LAP precludes its interaction either with cellular receptors, which would result in its degradation, or with other proteins that might mask the epitopes detected by antibodies to amino acids 1–30 of the N-terminal domain. In contrast, the LAP expressed from the transgene construct may remain at the site of synthesis and secretion. For example, spleen contains very high levels of TGF-β_1 mRNA (20) and protein, as determined after acid–ethanol extraction (21), yet none can be detected with anti-LC-(1–30) or anti-CC-(1–30) (20), suggesting that the mature TGF-β in this tissue is complexed to a binding protein so as to block the 1–30 epitope.

Histopathology of HK1.TGF-β_1 Transgenic Skin. When the morphology of HK1.TGF-β_1 transgenic skin is compared with controls, three differences can be seen. The transgenics have fewer hair follicles, a slightly thinner interfollicular epidermis, and a more compact orthohyperkeratosis (Fig. 2 *C–F*). The counts of the mean number of cross sections of hair follicles are shown in Table 1. The control epidermis had ≈38 cross sections of hair follicles per mm, whereas the transgenic mice had only 11 follicles per mm, a decrease of 71%. Histologically, the transgenic epidermis appeared thinner than its corresponding control, but counts of the number of nucleated epidermal cells did not differ. Ultrastructurally, the number and appearance of cell organelles, including intermediate filaments and keratohyalin granules, did not differ from controls. The orthohyperkeratosis is shown in Fig. 2*E* as a slightly darker staining, more compact cornified cell layer. At the ultrastructural level, where individual cornified cells are countable, the control mice had ≈16 cornified cell layers, whereas the transgenic mice had ≈24, an increase of 33%. Histologically, the transgenic dermis looked like its corresponding control, in that the number and position of fibroblasts and the amount of collagen were the same, and neither showed an influx of lymphocytes. At the ultrastructural level, no differences in fibroblastic cell organelles or collagen bundles or bands were seen.

Table 1. Number of cross sections of hair follicles in HK1.TGF-β_1 transgenic mice as compared with controls

Animal	Hair follicles, mean no. per mm length epidermis (SD)
Normal	
4164-7	48.8 (4.1)
4174-8	37.5 (2.3)
4186-6	28.4 (4.0)
Mean	38.2 (8.3)
Transgenic	
4164-9	11.0 (1.6)
4174-7	12.4 (0.8)
4186-5	9.2 (1.2)
Mean	10.9 (1.3)

HK1.TGF-β_1 Transgenic Mice Exhibit Aberrant Keratin Expression and Growth Arrest of the Epidermis and Hair Follicles. In addition to morphological studies, immunofluorescent studies were done by using antibodies to K1, K6, K10, K14, filaggrin, and loricrin. We found no discernible differences between the transgenic and normal epidermis in expression of K1, K10, K14, filaggrin, or loricrin. However, a marked induction of K6 was found in the interfollicular epidermis of all four transgenic mice, whereas controls only exhibited K6 expression in hair follicles (Fig. 2 *G* and *H*). Although expression of K6 in the interfollicular epidermis is usually associated with hyperproliferation (22), the transgenic mice showed no hyperproliferative activity. In fact, almost no epidermal mitotic activity was found in organ culture using BrdUrd in the medium with subsequent labeling by an anti-BrdUrd antibody. This result was most evident in the animal with the most severe phenotype, whereas the control littermate showed normal labeling of hair follicles and interfollicular epidermis (Fig. 2 *I* and *J*). Control epidermis had ≈17 labeled nuclei per mm, whereas transgenic epidermis had only four labeled nuclei per mm, a decrease of 76%.

DISCUSSION

We have successfully targeted expression of TGF-β_1 to the epidermis of transgenic mice and observed a profound skin phenotype that confirms the role of TGF-β_1 as a potent inhibitor of epithelial cell proliferation. Because previous attempts to produce mice over-expressing TGF-β had resulted in embryonic lethality (23), we used an epidermal-specific vector, which is expressed relatively late during mouse development, day 15, when the embryonic skin initially stratifies (15). To circumvent potential problems with endogenous mechanisms that regulate TGF-β expression and activation, we targeted a mutant form of TGF-β_1, which is constitutively active (16), and deleted 5′ and 3′ noncoding sequences from the TGF-β_1 cDNA, which have been implicated in posttranscriptional regulation (24).

The shiny, stretched skin of the HK1.TGFβ_1 pups immediately distinguished them from their unaffected littermates. On the basis of their macroscopic appearance, we expected that the histological examination would reveal a markedly thinner epidermis. However, this was not the case. Although the phenotypic epidermis exhibited a compact orthohyperkeratosis and appeared thinner, the number of nucleated epidermal cells did not differ from controls (Fig. 2 *E* and *F*). The clue for understanding the discrepancy between the severe macroscopic pathology and the lack of an underlying histopathological correlation comes from the pulse-labeling studies with BrdUrd (Fig. 2 *I* and *J*). The proliferative capacity of the basal epidermal cells was almost completely shut down, possibly as early as day 15 of embryogenesis, the time at which the K1 promoter is thought to become active. Such growth arrest of the epidermis, while the remainder of the embryo continued growing, produced phenotypic pups resembling over-inflated balloons at the time of birth (Fig. 1*B*).

We also observed a decrease (≈70%) in the number of hair follicles present in transgenic skin. Hair buds begin to form at day 14–15 during mouse development (25), essentially at the same time that our targeting vector is expressed. The lack of complete suppression of hair follicle formation is not surprising because the formation of some follicles may have been initiated before accumulation of inhibiting levels of TGF-β_1. However, by the time of birth, follicles that had initially formed failed to exhibit demonstrable proliferative activity. These observations are consistent with previous *in vitro* studies that showed that TGF-β_1 and TGF-β_2 could inhibit DNA synthesis in hair follicle organoids grown in a three-dimensional culture system (26).

Because we had previously observed induction of K6 in the hyperproliferative epidermis of transgenic mice expressing the v-Ha-*ras* oncogene or TGF-α (15, A. M. Dominey, X. J. Wang, T. A. Gagne, D.B., J.A.R., D. A. Greenhalgh, D.R.R., unpublished work), we were surprised to find K6 expression in the growth-arrested epidermis of the TGF-β_1 transgenic mice. Synthesis of the K6/K16 pair of keratins was initially thought to occur only under hyperproliferative conditions (22). However, a more recent study by Sun and coworkers (27) suggested that although hyperproliferation is usually accompanied by K6/K16 expression, the reverse is not always true (27). In fact, they were able to show continued synthesis of K6/K16 in cultures of rabbit corneal epithelial cells after treatment with DNA synthesis inhibitors. They suggested that synthesis of K6/K16 may indicate an alternative pathway of keratinocyte differentiation that occurs under conditions that are nonpermissive for keratinocytes to express their normal, differentiation-related keratin pairs. Mansbridge and Hanawalt (28) have suggested that such conditions exist during wound healing—i.e., regenerative maturation vs. normal maturation—and that TGF-β may play a major role in regulating this process. In support of this notion, they were able to demonstrate that TGF-β could, in fact, increase synthesis of K6/K16 when added to cultured human keratinocytes (28). Therefore, our results, taken together with the data summarized above, suggest that the induction of K6/K16 can occur in response to factors that exist under various pathological conditions and not strictly those present during hyperproliferation.

Given that TGF-β induced fibrosis and angiogenesis when injected s.c. in newborn mice (8), we anticipated that our TGF-β transgenic mice would exhibit a similar dermal phenotype if TGF-β produced in the epidermis was transported across the basement membrane and accumulated in the dermis. However, no obvious changes were observed. A more definitive analysis will be required to support our initial findings. If such a restricted expression of TGF-β in the epidermis does not produce unwanted side effects in the dermis, then these observations may have important implications for therapeutic applications. Although our transgenic study clearly shows that constitutive over-expression of TGF-β_1 suppresses epidermal cell proliferation and adversely affects skin development, regulated expression of active forms of TGF-β_1 in the epidermis could be beneficial in the treatment of hyperproliferative skin diseases. This approach could also be useful in the treatment of inflammatory skin diseases because recent reports describing the targeted disruption of the TGF-β_1 gene have confirmed its vital role in modulating the inflammatory response (29, 30).

We thank both Andrew Geiser and Paturu Kondaiah for making the TGF-β_1 construct. They also thank Ms. Janelle Laminack for invaluable secretarial assistance. K.S. was the recipient of an AIDS Research Fellowship of the Deutsches Krebsforschungszentrum. This work was supported, in part, by National Institutes of Health Grant HD25479 (D.R.R.) and a grant from the Texas Advanced Technology Program ATP004949048 (D.R.R.), and a Max Planck Research Award from the Alexander von Humboldt Foundation (T.K. and D.R.R.).

1. Roberts, A. B. & Sporn, M. B. eds. (1990) in *Handbook of Experimental Pharmacology* (Springer, Heidelberg), pp. 419–472.
2. Massaque, J. (1990) *Annu. Rev. Cell Biol.* **6,** 597–646.
3. Soma, Y. & Grotendorst, G. R. (1989) *J. Cell. Physiol.* **140,** 246–253.
4. Jutley, J. K., Cunliffe, W. J. & Wood, E. J. (1991) *J. Invest. Dermatol.* **96,** 1004.
5. Shipley, G. D., Pittelkow, M. R., Willie, J. J., Scott, R. E. & Moses, H. L. (1986) *Cancer Res.* **46,** 2068–2071.
6. Coffey, R. J., Sipes, N. J., Bascom, C. C., Graves-Deal, R., Pennington, C. Y., Weissman, B. E. & Moses, H. L. (1988) *Cancer Res.* **48,** 1596–1602.
7. Pittelkow, M. R., Coffey, R. J. & Moses, H. L. (1988) *Ann. N.Y. Acad. Sci.* **548,** 211–224.
8. Roberts, A. B., Sporn, M. B., Assoian, R. K., Smith, J. M., Roche, N. S., Wakefield, L. M., Heine, U. I., Liotta, L. A., Falanga, V., Kehrl, J. H. & Fauci, A. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4167–4171.
9. Flanders, K. C., Thompson, N. L., Cissel, D. S., Ellingsworth, L. R., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell Biol.* **108,** 653–660.
10. Pelton, R. W., Dickinson, M. E., Moses, H. L. & Hogan, B. L. M. (1990) *Development (Cambridge, UK)* **110,** 609–620.
11. Pelton, R. W., Hogan, B. L. M., Millder, D. A. & Moses, H. L. (1990) *Dev. Biol.* **141,** 456–460.
12. Millan, F. A., Denhez, A. F., Konaiah, P. & Akhurst, R. J. (1991) *Development (Cambridge, UK)* **111,** 131–144.
13. Schmid, P., Cox, D., Bilbe, G., Maier, R. & McMaster, G. K. (1991) *Development (Cambridge, UK)* **111,** 117–130.
14. Pelton, R. W., Saxena, B., Jones, M., Moses, H. L. & Gold, L. I. (1991) *J. Cell Biol.* **115,** 1091–1105.
15. Greenhalgh, D. A., Rothnagel, J. A., Quintanilla, M. I., Orengo, C. C., Gagne, T. A., Bundman, D. S., Longley, M. A. & Roop, D. R. (1993) *Mol. Carcinog.* **7,** 99–110.
16. Brunner, A. M., Marquardt, H., Malacko, A. R., Lioubin, M. N. & Purchio, A. F. (1989) *J. Biol. Chem.* **264,** 13660–13664.
17. Greenhalgh, D. A., Rothnagel, J. A., Wang, X.-J., Quintanilla, M. I., Orengo, C. C., Gagne, T. A., Bundman, D. S., Longley, M. A., Fisher, C. & Roop, D. R. (1993) *Oncogene*, in press.
18. Yuspa, S. H., Hawley-Nelson, P., Stanley, J. R. & Hennings, H. (1980) *Trans. Proc.* **12,** Suppl. 1, 114–122.
19. Wakefield, L. M., Winokur, T. S., Hollands, R. S., Christopherson, K., Levinson, A. D. & Sporn, M. B. (1990) *J. Clin. Invest.* **86,** 1976–1984.
20. Thompson, N. L., Flanders, K. C., Smith, J. M., Ellingsworth, L. R., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell Biol.* **108,** 661–669.
21. Danielpour, D. (1993) *J. Immunol. Methods* **158,** 17–25.
22. Weiss, R. A., Eichner, R. & Sun, T.-T. (1984) *J. Cell Biol.* **98,** 1397–1406.
23. Sporn, M. B. & Roberts, A. B. (1992) *J. Cell Biol.* **119,** 1017–1021.
24. Kim, S.-J., Park, K., Koeller, D., Kim, K. Y., Wakefield, L. M., Sporn, M. B. & Roberts, A. B. (1992) *J. Biol. Chem.* **267,** 13702–13707.
25. Davidson, P. & Hardy, M. G. (1952) *J. Anat. Physiol.* **86,** 342–356.
26. Weinberg, W. C., Brown, P. D., Stetler-Stevenson, W. G. & Yuspa, S. H. (1990) *Differentiation* **45,** 168–178.
27. Schermer, A., Jester, J. V., Hardy, C., Milano, D. & Sun, T.-T. (1989) *Differentiation* **42,** 103–110.
28. Mansbridge, J. N. & Hanawalt, P. C. (1988) *J. Invest. Dermatol.* **90,** 336–341.
29. Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N. & Doetschman, T. (1992) *Nature (London)* **359,** 693–699.
30. Kulkarni, A. B., Huh, C.-G., Becker, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M. & Karlsson, S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 770–774.